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Human leukocyte antigen-associated drug hypersensitivity

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A growing number of associations between adverse drug reactions and alleles of the *human leukocyte antigen (HLA)* genes are now known. Although several models have been proposed to explain these associations, an underlying molecular basis has only recently been described. The associations between *HLA-B*57:01* and abacavir hypersensitivity syndrome, and *HLA-B*15:02* and carbamazepine-induced bullous skin disease have provided new insights into the mechanism associated with hypersensitivity reactions to these drugs. Here we discuss recent evidence that small molecules can interact with specific HLA to distort self-peptide presentation leading to autoimmune-like drug hypersensitivities that potentially provide clues to the mechanisms underlying other immunopathologies.

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Introduction

Adverse drug reactions (ADR) inflict a significant toll on the health care system, as one of the leading causes of death amongst U.S. hospital patients [1]. There are two main categories of ADR, the predictable (Type A) ADR, linked with the drug's pharmacological activity and the idiosyncratic or non-predictable (Type B) ADR, often caused by drug-induced immune activation. In recent years the quest to find predictive markers of type B ADR has led to the discovery of numerous ADR associations with alleles of the *human leukocyte antigen (HLA)* genes [2].

Facilitated by genome wide association studies [3], this list is growing constantly (Table 1), highlighting a need to understand the mechanisms driving these associations. One subtype of idiosyncratic ADR is delayed type hypersensitivity (DTH), also known as T cell mediated drug hypersensitivity. DTH occurs after a minimum of 3–4 days drug exposure, resolves on cessation of drug use and occurs more rapidly on drug re-introduction [4–6]. This aligns closely with pathogen-induced T cell priming and reactivation on primary and secondary exposure. T cell involvement is further implicated by the recovery of drug-responsive T cells from patients [7–9]. Together these observations have led to the proposal that ADR associated with specific alleles of the *HLA* are caused by a unique functional involvement of the particular *HLA* gene product. Here we review recent progress in our understanding of the functional relationship between these ADR and their associated *HLA* alleles.

Affects of HLA polymorphism on peptide presentation

The *HLA* genes are among the most polymorphic in the human genome; the *HLA-B* locus alone possesses over 1000 expressed allotypes [10,11]. The immunopeptidome (the peptides bound to and presented by the HLA) is affected by these polymorphisms in multiple ways. Firstly, polymorphisms alter the shape and electrochemistry of pockets within the peptide-binding groove that subsequently determine the repertoire of peptides that can bind to a given HLA molecule. For HLA class I, these pockets are denoted A, B, C, D, E and F (Figure 1a), and for class II, P1, P4, P6, P7 and P9 (not shown). As a result, each HLA allotype possesses a “peptide-binding motif”, which can be characterised via sequencing of peptides bound within the cleft [12]. For example, *HLA-B*57:01* ligands are anchored within the peptide binding cleft predominantly using Ser or Thr residues at position 2 (P2) of the peptide and predominantly Trp, or less frequently Phe (Trp > Phe), at the C-terminus. The side chains of these residues extend into the B and F pockets respectively. In comparing *HLA-B*57:01* to *HLA-B*57:03*, the two amino acid substitutions that distinguish these allotypes (Asp114Asn and Ser116Tyr in the vicinity of the F pocket), shift the C-terminal bias of the peptide from Trp > Phe to Phe > Trp, and alter the overall peptide repertoire (Figure 1b) [13•].

Secondly, polymorphisms also affect the ability of HLA molecules to interact with components of the peptide loading machinery. The chaperone tapasin interacts with

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the HLA-I during peptide loading to cause the exchange of low affinity peptides for higher affinity peptides. Single amino acid substitutions at either 114 or 116 are sufficient to allow peptide loading independent of tapasin [14,15], resulting in differing kinetics of HLA egress from the endoplasmic reticulum (ER) in the presence of viral inhibitors of tapasin, as well as impacting the immunopeptidome [16]. Although polymorphism of HLA alleles has been established to drive peptide ligand diversity [13^{••},17–19], there has been limited exploration of its impact on interactions with small molecule drugs.

Drugs stimulating the immune system

It has long been established that many drugs, such as penicillins, have the ability to covalently modify proteins, either directly, or via metabolic intermediates. This can result in stimulation of antibody responses, causing antibody mediated drug hypersensitivities, as well as the presentation of haptenated peptides to T cells via the HLA [20,21]. The generation of haptenated peptides is proposed to be dependent on covalent modification of the source protein, followed by proteasome mediated degradation and antigen processing. Loaded onto the HLA,

Table 1

Adverse drug reactions associated with the HLA (updated from [2])

	Drug	Adverse reaction	HLA association(s)	OR/RR
HLA-I associations	Abacavir	AHS	B*57:01 [23 [*] ,24 [*] ,43,44]	>500
	Allopurinol	SJS/TEN and HSS	B*58:01 [45–47]	>800
	Carbamazepine	SJS/TEN	B*15:02 in several Asian populations [33 [*] ,34,35,39]	>1000
			B75 family members found in reactions in absence of HLA-B*15:02:	
			B*15:08 [35]	
			B*15:11 [40,41]	
			B*15:21 [40]	
			A*31:01 in European population [48]	25.9
			A*31:01 in Japanese population [38]	33.9
		MPE	A*31:01 in Han Chinese population [39]	17.5
			A*31:01 in European population [48]	8.3
		HSS	A*31:01 in European population [48]	12.4
		cADR (includes SJS/TEN, MPE and erythema multiforme and HSS)	A*31:01 in Japanese population [38]	9.5
	Feprazone	FDE	B22 [49]	48
	Flucloxacillin	DILI	B*57:01 [50]	80
	Levamisole	Agranulocytosis	B27 [51]	N.C.
	Oxicam	SJS/TEN	A2	6.6
			B12 [52]	4.3
	Methazolamide	SJS/TEN	A*59:01 [53]	~250
	Phenytoin	SJS/TEN	B*15:02 [34,54]	18.5
	Sulfamethoxazole	FDE	A30 [55]	6.2
	Sulfonamides (including sulfamethoxazole)	TEN	A29	8.4
			B12	10.6
			DR7 [56]	4.9
	Ticlopidine	DILI	A*33:03[57]	13
HLA-II associations	Aspirin	Asthma	DPB1*03:01 [58,59]	4.4/8.3
		Urticaria	DRB1*13:02-DQB1*06:09 haplotype [60]	2.5 [*]
			DR4 [61]	3.4 [*]
	Hydralazine	Systemic lupus erythematosus		5.5
	Lapatinib	DILI	DRB1*07:01-DQA1*02:01-DQB1*02:02 haplotype [62]	6.9 ^a
				9.0 ^a
	Lumiracoxib	DILI	DRB1*15:01-DQB1*06:02-	6.9 ^a
			DRB5*01:01-DQA1*01:02	7.5 ^a
			haplotype [63]	6.9 ^a
				7.2 ^a
	NSAIDs	Anaphylactoid and cutaneous reactions	DR11 [64]	6.3 ^a
	Ximelagatran	DILI		7.3
			DRB1*07-DQA1*02 [65]	4.4

Table 1 (Continued)

	Drug	Adverse reaction	HLA association(s)	OR/RR
Mixed associations	Aminopenicillins	HSS	A2, DRw52 [66]	6.7, 9.3
	Clozapine	Agranulocytosis	B38	50
			DR4	23.3
			DR2 [67]	6.0
	Co-amoxiclav (amoxicillin-clavulanate)	DILI	A*02:01-B*07:02-DRB1*15:01-DQB1*06:02 haplotype in Northwestern European Population [68]	13
			A*02:01-B*07:02-DRB1*15:01-DQB1*06:02 haplotype in Spanish Population [68]	20
			A*02:01-B*18:01 haplotype in Spanish Population [68]	6.4
			B8, DR3 [69]	N.C.
	d-Penicillamine	Proteinuria	B8, DR3 [69]	N.C.
	Gold Sodium thiomalate	Proteinuria	B8, DR3 [69]	N.C.
	Nevirapine	Cutaneous reactions	Cw4 [70], B*35:05 [71] in Thai population	N.C., 30
		HSS	DRB1*01:01 in French population [72]	N.C.
			Cw8-B14 in Sardinian population [73]	N.C.
			Cw8 in Japanese population [74]	N.C.
		Rash-associated hepatitis	DRB1*01:01 in Australian population [75]	N.C. – affected by CD4 T cell count

OR – odds ratio, RR – relative risk, N.C. – not calculated, DILI – drug induced liver injury, FDE – fixed drug eruption.

^a Odds ratios for individual alleles of the extended haplotype.

these peptides generate novel, immunogenic complexes which traffic to the cell surface [4,22].

It was therefore postulated that HLA/ADR associations were caused by the generation of drug haptenated peptides uniquely able to bind the associated HLA allotype. However, recent investigations into T cell stimulation in abacavir hypersensitivity syndrome (AHS), mediated by HLA-B*57:01, have revealed a completely novel mechanism for drug-induced T cell activation.

Abacavir hypersensitivity syndrome and drug-induced changes in peptide presentation

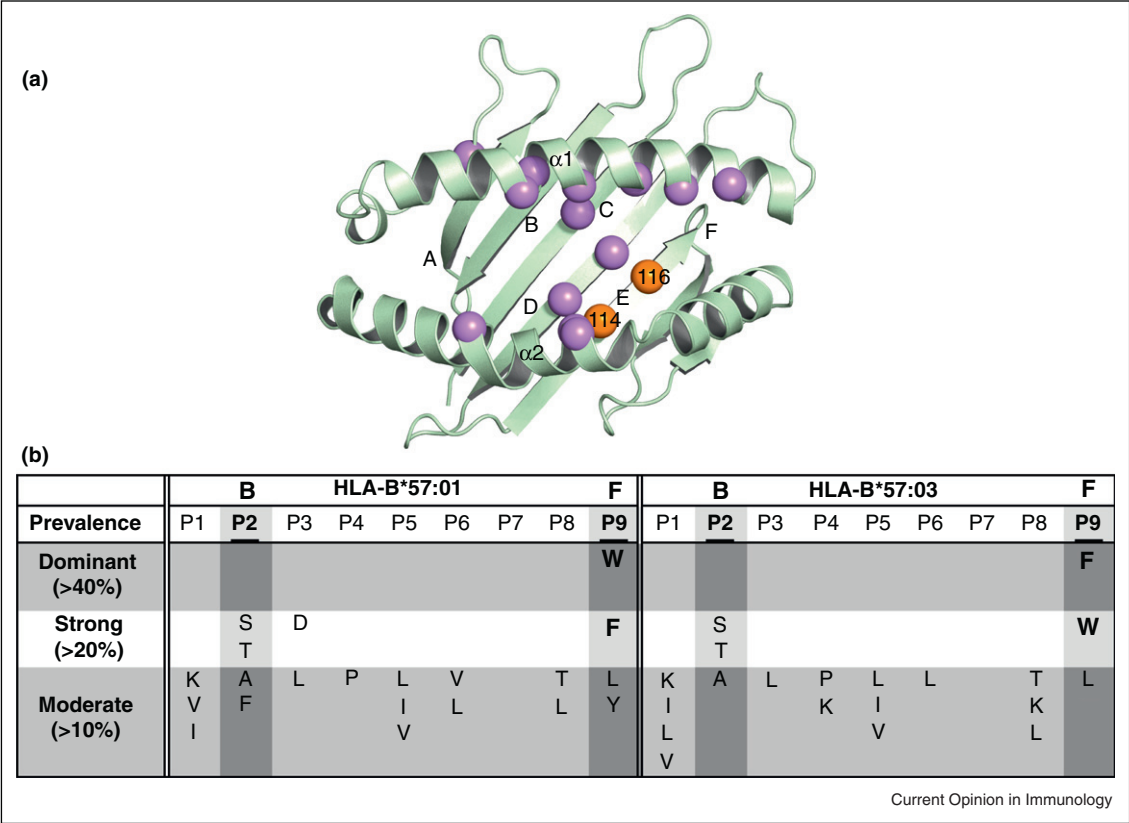
Abacavir (originally 1592U89) is an antiretroviral drug used to treat Human Immunodeficiency virus type I (HIV-I) infection. In 2002, two independent laboratories identified an association between AHS and the HLA class I allele, *HLA-B*57:01*, showing that 100% of individuals experiencing AHS possessed *HLA-B*57:01* [8,23[•],24[•],25[•]]. With an odds ratio greater than 500, this is one of the most compelling HLA/ADR associations identified (Table 1).

CD8⁺ T cell infiltrates are present in AHS-associated rash and patch testing [8] and drug-specific CD8⁺ T cell responses could be elicited from peripheral blood mononuclear cells from both AHS patients and abacavir-naïve, *HLA-B*57:01*⁺ blood donors [26^{••}]. These T cells responded to abacavir-treated antigen presenting cells (APCs) expressing HLA-B*57:01 but not closely related allotypes differing by just a few amino acids [26^{••}]. Even a

single residue difference in the F pocket of HLA-B*57:01 was sufficient to completely abrogate formation of the immunogenic complex. Furthermore antigen presentation was severely impacted by inhibition of the transporter associated with antigen processing (TAP) or tapasin deficiency.

Recently three separate studies showed perturbation of the HLA-B*57:01 peptide binding motif in the presence of abacavir [13^{••},27^{••},28]. Whilst peptides from untreated cells showed the standard HLA-B*57:01 motif (Figure 1b), those from cells cultured with abacavir showed a significant increase in peptides with Ile/Leu occupying the C-terminal anchor position. Novel self-peptides constituted approximately 20–25% of isolated peptides, indicating a huge alteration in self-presentation for abacavir loaded HLA-B*57:01. This aligns well with the polyclonal nature of the resulting T cell response, implying the response is mediated by an array of these novel antigens [13^{••}]. No change in the peptide-binding motif was seen with closely related allotypes HLA-B*57:03 (Asp114Asn and Ser116Tyr), nor HLA-B*58:01 (Met45Thr, Ala46Glu, Val97Arg and Val103-Leu) indicating minimal drug induced alterations in antigen processing and cellular protein expression [13^{••}]. Additionally, screening of randomised peptide libraries showed that peptides with Val or Ile at the C-terminus had enhanced affinity for HLA-B*57:01 when abacavir was present, explaining the increased abundance of peptides of this nature within the abacavir distorted repertoire [27^{••}].

Figure 1



Anchor pockets within the HLA and peptide binding motif. Cartoon diagram of the HLA-B*57:01 antigen binding cleft, showing the locations of the anchor pockets A–F. The locations of polymorphic residues that contribute to the architecture of the anchor pockets are highlighted in magenta and the polymorphic residues 114 and 116, which differ between HLA-B*57:01 and HLA-B*57:03 are shown in orange. The peptide binding motif of HLA-B*57:01 and HLA-B*57:03 (adapted from [13^{••}]), depicting residues most prevalent at P1 to P9 of peptide ligands of 9 amino acids length. Primary anchor positions within the peptide P2 and P9 are underlined, and occupy the B and F pocket respectively. Prevalence refers to the proportion of peptides within the dataset possessing the stated residue at the stated location. The major difference in motif between HLA-B*57:01 and HLA-B*57:03 at P9 is shown in bold.

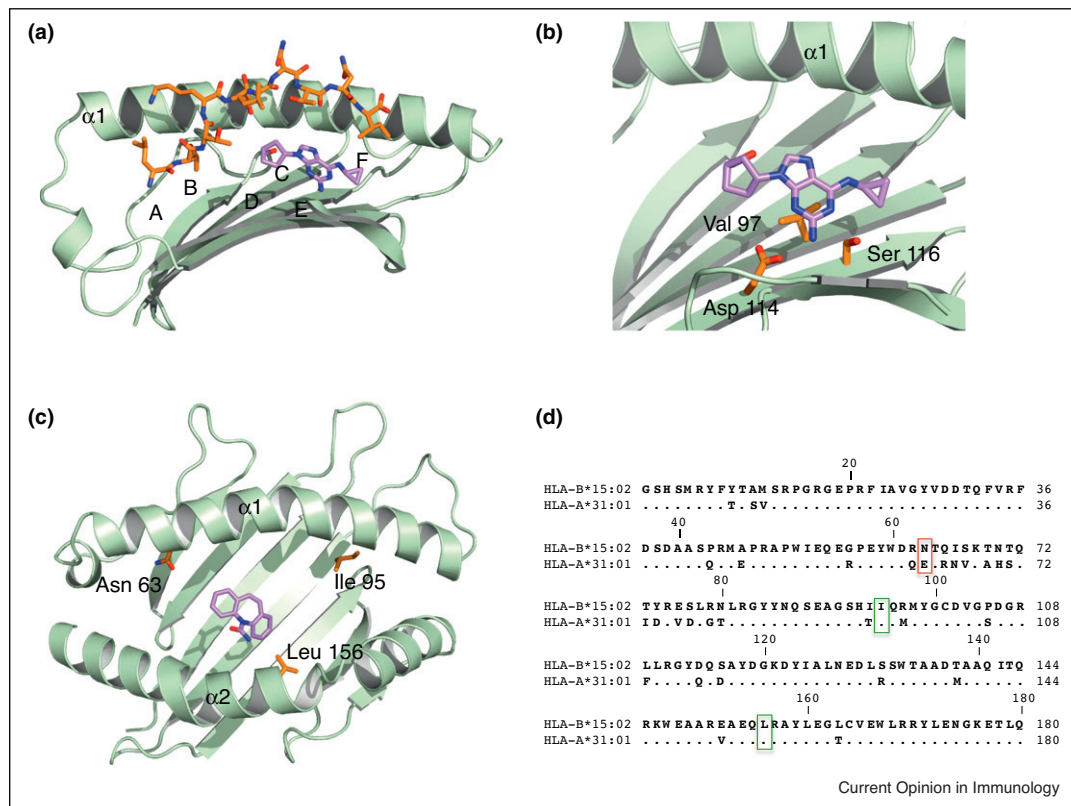
Determination of the structure of HLA-B*57:01:abacavir:peptide complexes showed abacavir bound within the peptide-binding cleft, buried underneath the C-terminal region of the peptide [13^{••},27^{••}]. Hidden from direct view of T cells, but changing the landscape of the HLA groove, abacavir thus altered the nature of the peptides that could be accommodated by HLA-B*57:01 (Figure 2a). Furthermore, interactions between abacavir and HLA-B*57:01 involved several polymorphic residues, indicating why HLA-B*57:03 and HLA-B*58:01 cannot bind abacavir efficiently (Figure 2b) [13^{••},27^{••}].

It is currently unclear when and how abacavir is incorporated into the HLA-B*57:01:peptide complex. Reduced T cell stimulation in the absence of tapasin and TAP activity in the APC [26^{••}]; evidence that abacavir can permeate the ER [29]; the appearance of novel self peptides within the immunopeptidome upon abacavir treatment [13^{••},27^{••},28]; and increased antigenicity of

APC incubated with abacavir over time, all point towards the ER as the primary location for abacavir loading. However there are also reported instances of T cell stimulation occurring much more rapidly than can be explained by export of *de novo* HLA:peptide complexes from the ER, suggesting some level of binding at the cell surface, potentially altering the conformation of existing HLA:peptide complexes [30].

Alloreactivity caused by potent single residue mismatches such as between HLA-B*44:02 and HLA-B*44:03 highlights the capacity for T cell response driven by modifications buried within the antigen binding cleft. In this instance the single, buried, amino acid polymorphism, Asp156Leu, affects both peptide repertoire and the mode of display of identical peptides, resulting in large T cell alloresponses across these differences [17,31,32]. Similarly, T cell stimulation in AHS may not only be caused by presentation of novel self peptides (altered

Figure 2



Interaction of Abacavir and HLA-B*57:01. A side view of the HLA-B*57:01-abacavir-LTTKLTNTNI complex showing the floor of the peptide-binding groove (PDB accession number 3VRJ). Abacavir is shown in magenta, HLA-B*57:01 in green and LTTKLTNTNI in orange. The α_2 helix has been removed for clarity. The pockets A-F are marked, as is the α_1 helix. A close up view of abacavir, highlighting in orange how the polymorphic residues in non-AHS associated HLA-B*57:03 (Asp114Asn, Ser116Tyr) and HLA-B*58:01 (Val97Arg) allotypes would prevent abacavir binding. Ribbon diagram of the HLA-B*15:02 antigen binding cleft (based on structure of HLA-B*15:01, PDB accession 1XR8), showing the locations of residues implicated in binding of CBZ. 63Asn, 95Ile and 156Leu are shown. CBZ (magenta) has been placed in the cleft using *in silico* docking [13**]. Amino acid sequence alignment of the α_1 and α_2 domains of HLA-B*15:02 and HLA-A*31:01. Key residues implicated in binding of CBZ by HLA-B*15:02 are marked by boxes (green for 95Ile and 156Leu, red for 63Asn). Adapted from [13**].

repertoire in the presence of abacavir), but also by altered conformation of existing HLA-B*57:01:self-peptide complexes (Figure 3). Considering the parallels in symptoms between AHS and graft-versus host disease, a model of T cell stimulation by generation of a “pseudo allo-HLA” provides a compelling case for the proposed pathogenesis of AHS.

Investigations of other HLA/ADR associations

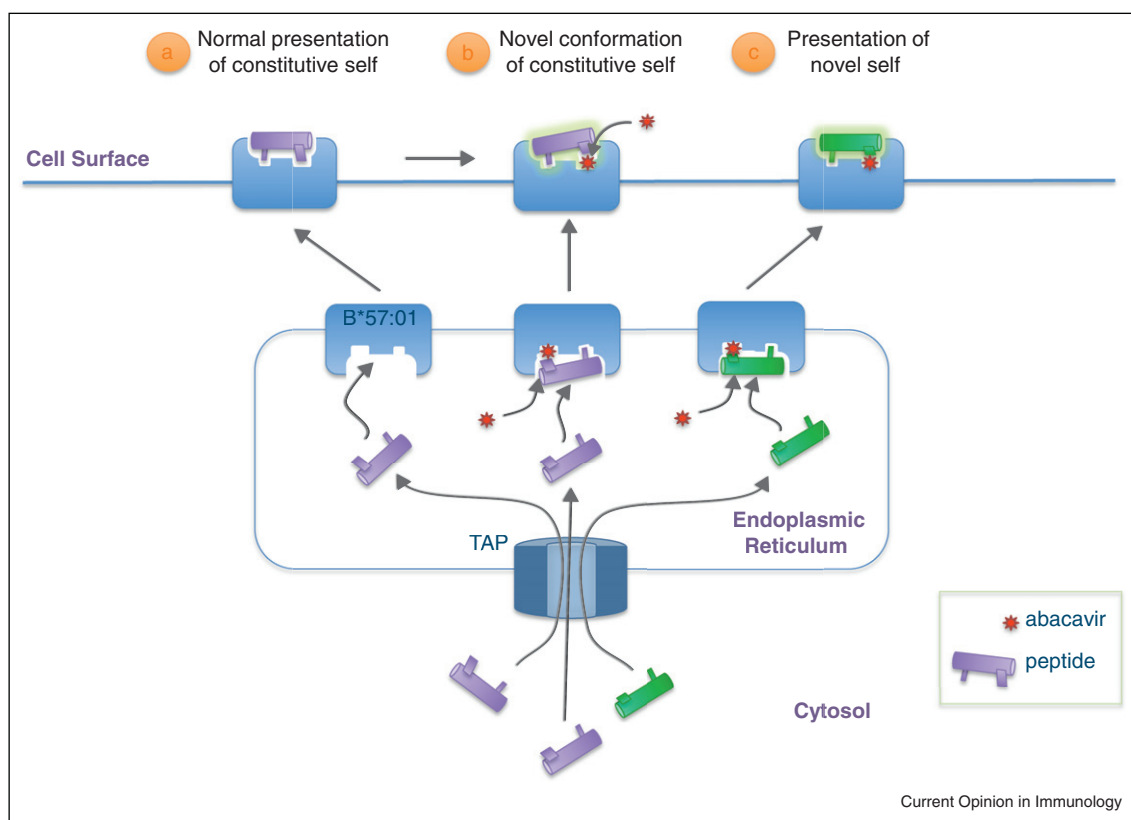
The widely used antiepileptic and mood modifier, carbamazepine (CBZ), is responsible for a diverse range of cutaneous ADR (cARD) including hypersensitivity syndrome (HSS), maculopapular exanthema (MPE), Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). SJS and TEN are considered lesser and more severe manifestations of the same bullous skin disease, presenting as significant skin and mucosal detachment, and showing clear CD8⁺ T cell involvement.

First discovered in the Han Chinese population, and later observed in Thai and Indian populations, CBZ induced SJS/TEN is associated with *HLA-B*15:02* [33*,34,35]. As with AHS and *HLA-B*57:01*, the association between *HLA-B*15:02* and CBZ-SJS/TEN is incredibly strong (odds ratio > 1000) suggesting that possession of *HLA-B*15:02* is one of the few factors necessary for disease manifestation in these populations.

The T cell response to CBZ appears to contrast that of abacavir, apparently recruiting a more restricted T-cell repertoire [36], suggesting a limited array of immunogenic HLA-B*15:02:peptide:CBZ complexes. Consistent with this, the impact of CBZ on the peptide repertoire of HLA-B*15:02 is less marked than that of abacavir on HLA-B*57:01 [13**]. Recent studies have also suggested that the generation of immunogenic HLA complexes may occur at the cell surface and can be formed with both CBZ

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Figure 3



Models for generation of immunogenic HLA-B*57:01:abacavir:peptide complexes. In the absence of abacavir, HLA-B*57:01 presents peptides generally of 9–10 amino acids length, predominantly terminating with a Trp or Phe residue, and a smaller number which terminate with Leu, Tyr and Val. These are bound within the ER and presented at the cell surface, where they are tolerated by circulating T cells (a). Immunogenic HLA-B*57:01:abacavir:peptide complexes may be generated either via (b) incorporation of abacavir with peptides of the constitutive repertoire, either at the cell surface, or within the ER, such that the peptides are presented with an altered conformation, or (c) the stabilisation of “novel self” ligands absent from the constitutive repertoire but favoured in the presence of abacavir.

and its metabolite CBZ-10,11-epoxide [37]. Hence, the mechanism of CBZ-mediated hypersensitivity may be more complex than for abacavir.

Interestingly, in 2011, associations between CBZ induced cARD in Caucasian and Japanese populations and *HLA-A*31:01* were reported [38]. This allele is also associated with CBZ-induced MPE in Han Chinese [39]. *HLA-B*15:02* and *HLA-A*31:01* differ greatly in amino acid sequence, however they share two of the three residues suggested to control the interaction of CBZ and *HLA-B*15:02* (95Ile and 156Leu but not 63Asn) [37] (Figure 2c and d). However, an Asn63Glu substitution, as exists between these allotypes, was seen to abrogate CBZ binding, suggesting that CBZ presentation by *HLA-A*31:01* may differ greatly to that mediated by *HLA-B*15:02* and other *HLA-B75* family members [35,37,40,41]. The elucidation of binding modes for CBZ by these allotypes using structural techniques is a logical next step in our quest to understand T cell stimulation in CBZ reactions.

In addition, a recent study by Michels *et al.* [42] used *in silico* docking techniques to directly target the anchor pockets of *I-A^{G7}*, the *HLA-II* homologue that predisposes NOD mice to autoimmune diabetes. *In vitro* testing of lead candidates showed molecules targeting either P1 or P6 inhibited activation of T cells by the insulin B9-23 epitope, whilst those targeting P9 increased T cell activation. Furthermore, some, but not all, of the P1 and P6 targeting molecules were able to inhibit presentation of higher affinity *I-A^{G7}* ligands, demonstrating differing peptide specificity [42]. The alteration of these responses is probably caused by either an inhibition of binding or an alteration in the mode of presentation of these peptides in the presence of the drug. This is reminiscent of the abacavir-modified repertoire of *HLA-B*57:01*, where constitutive self-ligands are displaced by other self-peptides able to co-occupy the cleft with abacavir. Whether the molecules identified by Michels *et al.* show a similar perturbation of the immunopeptidome, or any allotype specificity, remains to be seen.

Concluding remarks

It seems unlikely that the specific interaction between abacavir and HLA-B*57:01 is unique with respect to HLA/ADR associations. Attributes of drugs, such as systemic delivery, high plasma concentrations and prolonged half-lives, are ideal for agents that might engender immune recognition. Small size and molecular variability across the pharmacopeia, coupled with the extreme polymorphism and the ubiquitous expression of HLA molecules, creates a perfect setting for 'off-target' drug effects manifested as immune recognition, as evident from the growing list of HLA/ADR associations (Table 1). Whilst such interactions present an additional consideration during preclinical drug development, prior knowledge of HLA interactions may be used to guide lead optimisation. Cellular metabolism generates a plethora of small molecules that may be candidates for abacavir-like interaction with specific HLA. Whether naturally occurring small molecules interacting with the HLA might underlie HLA/autoimmune disease associations such as Bechet's disease and *HLA-B51*, Birdshot retinopathy and *HLA-A29* or ankylosing spondylitis and *HLA-B27*, remains an intriguing question.

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- This study was one of the first observations of the link between HLA-B*57:01 and abacavir hypersensitivity. The strength of the association was not fully realised as the result of less stringent phenotyping of AHS than was later employed by this group (see Ref. [25**]).

8 Antigen processing

24. Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, Lai E, Davies K, Handley A, Dow DJ *et al.*: **Genetic variations in HLA-B region and hypersensitivity reactions to abacavir.** *Lancet* 2002, **359**:1121-1122.

Independent of Mallal *et al.* (Ref. [23*]), this study was one of the first to demonstrate an association between *HLA-B*57:01* and AHS.

25. Mallal S, Phillips E, Carosi G, Molina J-M, Workman C, Tomazic J, Jagel-Guedes E, Rugina S, Kozlyev O, Cid JF *et al.*: **HLA-B*5701 screening for hypersensitivity to abacavir.** *N Engl J Med* 2008, **358**:568-579.

Using immunological patch testing, this study showed that 100% of individuals experiencing true AHS possessed *HLA-B*57:01*. Most importantly their work showed that screening for *HLA-B*57:01* before the initiation of abacavir treatment could significantly reduce the incidence of AHS.

26. Chessman D, Kostenko L, Lethborg T, Purcell AW, Williamson NA, Chen Z, Kjer-Nielsen L, Mifsud NA, Tait BD, Holdsworth R *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**:822-832.

This paper characterised the requirements for presentation of immunogenic abacavir complexes in AHS. Demonstrating that abacavir responsive T cells could be expanded from both AHS patients and abacavir-naïve *HLA-B*57:01*⁺ healthy blood donors, it further showed that activation could only be mediated by *HLA-B*57:01* and not closely related, non-AHS associated allotypes.

27. Ostrov DA, Grant BJ, Pompeu YA, Sidney J, Harndahl M, Southwood S, Oseroff C, Lu S, Jakoncic J, de Oliveira CAF *et al.*: **Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire.** *Proc Natl Acad Sci USA* 2012.

Utilising random peptide libraries, this study showed that peptides terminating with valine, alanine and isoleucine have increased affinity for *HLA-B*57:01* in the presence of abacavir. The nature of the interaction was elucidated using X-ray crystallography, showing (as in reference [13**]) that abacavir bound in the antigen binding cleft of *HLA-B*57:01*.

28. Norcross MA, Luo S, Lu L, Boyne MT, Gomarteli M, Rennels AD, Woodcock J, Margulies DH, McMurtrey C, Vernon S *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**:F21-F29.

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This study is the first report of the association between *HLA-B*15:02* and CBZ-SJS in the Han Chinese population. It showed that 100% of individuals experiencing CBZ-SJS were positive for *HLA-B*15:02*, whilst only 3% of CBZ-tolerant individuals possessed this allele. As a result numerous other groups have performed similar studies in other populations.

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