Drug Hypersensitivity and Human Leukocyte Antigens of the Major Histocompatibility Complex

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Keywords

adverse drug reactions, T cells, HLA polymorphism, delayed type hypersensitivity, hepatotoxicity

Abstract

The human leukocyte antigen (HLA) genes are the most polymorphic in the human genome and are critical in regulating specific immunity, hence their historical discovery as "immune response" genes. HLA allotypes are also implicated in unwanted immune reactions, including drug hypersensitivity syndrome, in which small therapeutic drugs interact with antigenic peptides to drive T cell responses restricted by host HLA. Abacavir, allopurinol, and carbamazepine are three commonly used drugs that cause a T cell–mediated hypersensitivity that is HLA linked, with each drug exhibiting striking specificity for presentation by defined HLA allotypes. Recent findings have begun to unearth the mechanistic basis for these HLA associations, and here we review recent advances in the field of HLA-associated drug hypersensitivities.

INTRODUCTION

ADR: adverse drug reaction

DHS: drug hypersensitivity syndrome

HLA: human leukocyte antigen

DTH: delayed type hypersensitivity

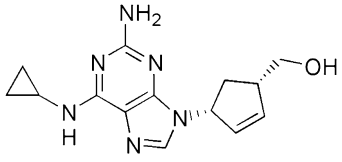
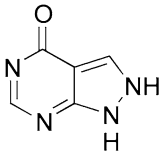
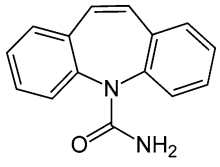
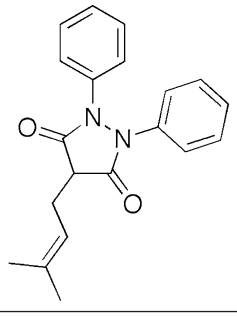
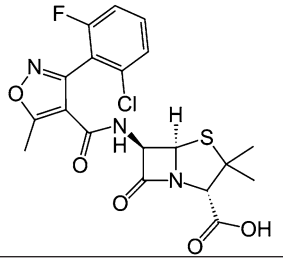
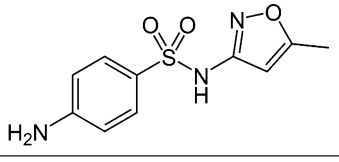
Despite growing pharmacological knowledge informing rational drug development, adverse reactions to medications remain commonplace. Adverse drug reactions (ADRs) are now one of the leading causes of morbidity and mortality in health care worldwide. In Australia, ADR-related hospital costs have been estimated at greater than $350 million annually (1); in the United States, the cost of drug-related morbidity and mortality has been estimated at US$136 billion annually (2), which is more than the total yearly cost of the country's cardiovascular or diabetic care. In 1996, 2.2 million Americans had adverse reactions, and 108,000 Americans died in hospitals from adverse reactions to drugs approved by the U.S. Food and Drug Administration (FDA) (3). The World Health Organization defines an ADR as "a response to a drug which is noxious and unintended, and which occurs at doses normally used or tested in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function." There are two major categories of ADR: the predictable (Type A) ADR, associated with the pharmacological activity of the drug, and the idiosyncratic (Type B) ADR, caused by an immune response to the drug (4). Variants of the latter are also known as drug hypersensitivity syndrome (DHS) or drug reaction with eosinophilia and systemic symptoms (DRESS). Although DHS constitutes only ~13% of all ADRs (5), it is typically more severe in nature and is clinically characterized by fever, rash, and failure of multiple organs including the liver, kidneys, lungs, and/or heart. In view of the serious outcomes of DHS and its etiology, a detailed knowledge of the immunological mechanisms leading to the pathogenesis associated with the Type B ADR is highly desirable for both prognosis and prevention of DHS and for optimal utilization of drug therapies. Here we review recent advances in the field of drug hypersensitivity reactions that are linked with particular human leukocyte antigen (HLA) alleles. (Also see the **Supplemental Material** associated with this article: **Supplemental Figures 1** and **2**; **Supplemental Table 1**; and a **Supplemental** section, "Non–Major Histocompatibility Complex Determinants of Drug Hypersensitivity." Follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**.)

Supplemental Material

DRUG HYPERSENSITIVITY SYNDROME

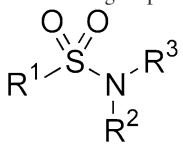
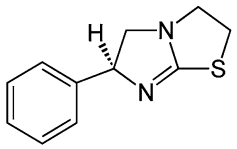
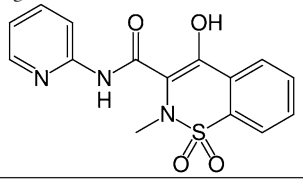
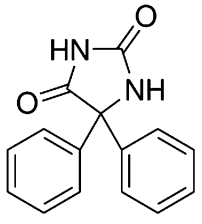
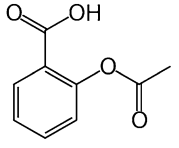
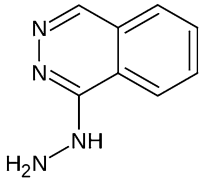
Although several hypotheses have been proposed to explain the immunopathogenesis of DHS, it is still unclear to what extent factors such as the host's gender, genetic polymorphisms, and age—or environmental agents such as diet, viral infections, and/or comorbidities—might also be involved. Genetic factors have been strongly implicated in the etiology of idiosyncratic drug reactions, including specific HLA allotypes, which are key proteins that regulate T cell–mediated immunity (**Table 1**). The HLA associations emerging with many ADRs are of greater strength than those with protection or susceptibility phenotypes in infectious disease. This is paradoxical because the main function of HLA molecules is to regulate protective immunity to microbes; this function is reasonably well understood, whereas the underlying mechanisms of HLA allele associations with ADRs remain undefined. Although the strength of association varies, the growing number of associations between ADR and HLA alleles (summarized **Table 1**) not only mandates some urgency in understanding this relationship but also may indicate general mechanisms in operation to mediate these reactions. One commonly reported idiosyncratic (Type B) ADR is delayed type hypersensitivity (DTH), a T cell–mediated, drug-specific recall response. DTH occurs after a minimum of 3–4 days of drug exposure, thus mirroring cell-mediated responses to viral infection; naive T cells are primed on initial exposure, and a memory pool is restimulated on repeat exposure. Accordingly, the reaction resolves on removal of drug therapy and occurs more rapidly on drug reintroduction (6–8). This strongly suggests that the observed associations

Table 1 Human leukocyte antigen (HLA) associations with drug hypersensitivities

	Drug	Structure	Adverse reaction	HLA association	Reference(s)
MHC class I associations	Abacavir		AHS	B*57:01	(43–45, 143)
	Allopurinol		SJS/TEN and HSS	B*58:01	(49, 51, 144)
	Carbamazepine		SJS/TEN	B*15:02	(46–48, 68)
	Feprazone		Fixed drug eruption	B22	(145)
	Flucloxacillin		Hepatitis	B*57:01	(114)
	Sulfamethoxazole[a]		Fixed drug eruptions	A30, B13, Cw6	(146)

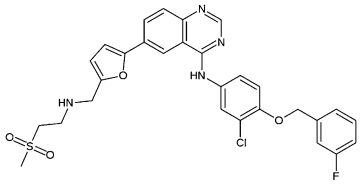
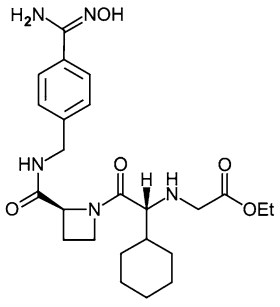
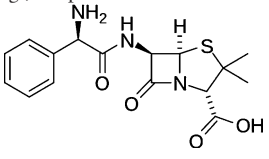
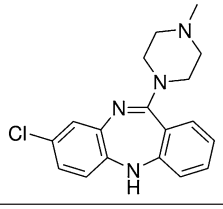
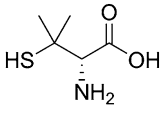
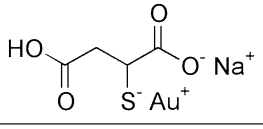
(*Continued*)

Table 1 (*Continued*)

	Drug	Structure	Adverse reaction	HLA association	Reference(s)
	Sulfonamides (including sulfamethoxazole)	Various structures, defining this functional group: 	TEN	A29, B12, DR7	(147)
	Levamisole		Agranulocytosis	B27	(148)
	Oxicam	e.g., Piroxicam 	SJS/TEN	A2, B12	(149)
	Phenytoin		SJS/TEN	B*15:02	(46, 78)
MHC class II associations	Aspirin		Asthma	DPB1*03:01	(81, 82)
			Urticaria	DRB1*13:02-DQB1*06:09	(80)
	Hydralazine		Systemic lupus erythematosus	DR4	(150)

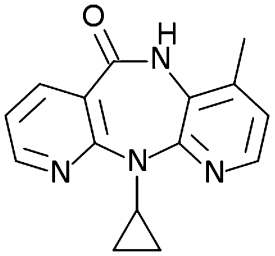
(*Continued*)

Table 1 (*Continued*)

	Drug	Structure	Adverse reaction	HLA association	Reference(s)
	Lapatinib		Hepatotoxicity	DRB1*07:01-DQA1*02:01-DQB1*0202/0203	(116)
	NSAIDs	Various structures	Anaphylactoid and cutaneous reactions	DR11	(151)
	Ximelagatran[a]		Elevated alanine aminotransferase	DRB1*07, DQA1*02	(117)
Mixed associations	Aminopenicillins	e.g., Ampicillin 	HSS	A2, DRw52	(152)
	Clozapine (Clozaril®)		Agranulocytosis	B38, DR4, DR2	(153)
	D-penicillamine		Proteinuria	B8, DR3, DR1	(154)
	Gold sodium thiomalate		Proteinuria	B8, DR3	(154)

(*Continued*)

Table 1 (*Continued*)

	Drug	Structure	Adverse reaction	HLA association	Reference(s)
	Nevirapine		Cutaneous reactions in Thai population	Cw4, B*35:05	(155, 156)
			HSS in Sardinian population	Cw8-B14	(140)
			HSS in Japanese population	Cw8	(139)
			Rash-associated hepatitis in Australian population	DRB1*01:01	(138)
			Cutaneous reactions in French population	DRB1*01:01	(157)

[a]Schematics of sulfamethoxazole and ximelagatran provided by Patricia Illing; reprinted with permission.
Abbreviations: HSS, hypersensitivity syndrome; MHC, major histocompatibility complex; NSAID, nonsteroidal anti-inflammatory drug; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

result from drug-induced T cell activation mediated by the associated HLA allotypes. This review discusses this hypothesis in relation to data regarding HLA-associated ADRs.

HUMAN LEUKOCYTE ANTIGEN MOLECULES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX: STRUCTURE AND FUNCTION IN CLASSICAL T CELL ACTIVATION

To understand how drugs may activate a T cell–mediated immune response, it is first necessary to understand the natural process of T cell activation. The *major histocompatibility complex* (*MHC*) genes, also known as the *HLA* genes in humans, encode cell-surface receptors that capture and present self- and pathogen-derived peptides to T cells as part of immune surveillance. Two types of the classical MHC molecules mediate this process: the MHC class I (MHCI) molecules, expressed by most nucleated cells, and the MHC class II (MHCII) molecules, expressed by specialized antigen-presenting cells (APCs). Additional signals are provided by APCs to tailor immune responses. MHCI and MHCII molecules are responsible for presenting peptides to $CD8^+$ (cytotoxic or killer) and $CD4^+$ (helper or regulatory) T cells, respectively. In humans, the classical MHCI molecule is encoded by three loci known as *HLA-A*, *HLA-B*, and *HLA-C*; the classical MHCII molecule is encoded by three loci known as *HLA-DR*, *HLA-DQ*, and *HLA-DP*. The genes encoding these complexes are linked within the MHC region on the short arm of chromosome 6 (see **Supplemental Table 1**). This region also contains genes for many other immune-associated proteins, including some nonclassical MHC molecules. Well-known patterns of linkage disequilibrium exist between combinations of alleles at linked loci (9). It is therefore possible that associations between ADRs and specific *HLA* alleles may result from linkage disequilibrium of the *HLA* allele in question with another causative genetic polymorphism such as a linked *HLA* gene within this area.

Supplemental Material

MHC: major histocompatibility complex

APC: antigen-presenting cell

The MHCI molecules comprise a polymorphic heavy chain (the α chain) consisting of three domains—α_1, α_2, and α_3—and a conserved single-domain light chain known as β_2-microglobulin (β_2m). The peptide-binding groove of MHCI is formed by the α_1 and α_2 domains, each of which

contributes 4 antiparallel β strands to a β sheet forming the base of the peptide-binding cleft, and an α helix, which together form the walls of the cleft (10). The binding cleft contains peptide-binding pockets that accommodate the side chains of certain amino acids of the bound peptide, anchoring them to the MHCI molecule (see **Supplemental Figure 1*a,b***). Other amino acid side chains have lesser interactions with the MHC and are more solvent exposed; thus, they are available for T cell receptor (TCR) contact (11). Both the α_3 domain and the β_2m possess immunoglobulin-like folds and are positioned below the peptide-binding groove, and α_3 attaches to the transmembrane domain to tether the structure to the cell surface (10).

TCR: T cell receptor

In the course of trafficking to the cell surface, the MHC must be stabilized by the loading of a peptide into the peptide-binding groove (12). In the case of MHCI molecules, this loading occurs in the endoplasmic reticulum (ER). Peptides derived from the natural, proteasome-mediated breakdown of intracellular proteins are transported into the ER via the transporter associated with antigen processing (TAP) that associates with the MHCI molecules as part of the peptide-loading complex (PLC). The chaperones tapasin and ERp57, also part of the PLC, help optimize the peptide loading through a process known as peptide editing, which favors higher-affinity peptides (13). Once loaded with a peptide, the MHC molecule is transported to the cell surface, where it becomes accessible for recognition by circulating T cells with cognate receptors (14). Peptides loaded by MHCI molecules generally consist of 9–11 amino acid residues, although larger peptides can be accommodated in some instances (15, 16). MHCI-peptide affinities can be in the nanomolar range, engendering a long half-life for these complexes on the cell surface (≥12 h) (17).

The MHCI-peptide complex is capable of interacting with specific TCRs of $CD8^+$ T cells, which are selected during their development for their ability to interact weakly with self-MHCI-bearing self-peptides but with insufficient strength to generate an immune response. In the event that a neopeptide, such as a virus-derived peptide, is presented by the MHCI under immunogenic conditions, T cells bearing complementary TCRs will be activated, stimulating an antigen-specific $CD8^+$ T cell response. Once activated, these cells proliferate and mediate effector functions such as cytotoxicity toward cells bearing the stimulatory peptide on their MHCI molecule. This helps prevent cells from producing "non-self" proteins such as viral proteins, while leaving unharmed normal cells (noninfected cells that lack expression of the stimulatory peptide). Thus, the MHCI can be seen as a surveillance system for identifying cells with aberrant protein expression that allows immune cell recognition.

Conversely, MHCII molecules are expressed by specialized APCs such as dendritic cells, B cells, and macrophages. MHCII molecules in these cells sample both endogenously and exogenously derived proteins after their digestion within the endosomal pathway. Peptide loading of the MHCII molecules occurs in a specialized, late endosomal vesicular structure known as the MHCII compartment, stabilizing MHCII molecules for transport to the cell surface. An MHCII molecule consists of two polymorphic chains, the α and β chains, each of which possesses two domains (see **Supplemental Figure 1*c,d***). The peptide-binding cleft is generated by the interaction of the α_1 and β_1 domains in a fashion analogous to the α_1 and α_2 domains of an MHCI molecule, whereas the MHCII α_2 and β_2 domains occupy similar positions to those of α_3 and β_2m in an MHCI molecule (18).

Peptides bound to the MHCII are often much longer than those bound to the MHCI (12–25 amino acids versus 9–11) (18, 19). MHCII molecules interact with $CD4^+$ T cells, and presentation of novel peptides by the MHCII under immunogenic conditions stimulates the proliferation of those possessing antigen-specific TCRs. This produces variable effector functions depending on other stimuli received from the APCs and on the type of immune challenge under way.

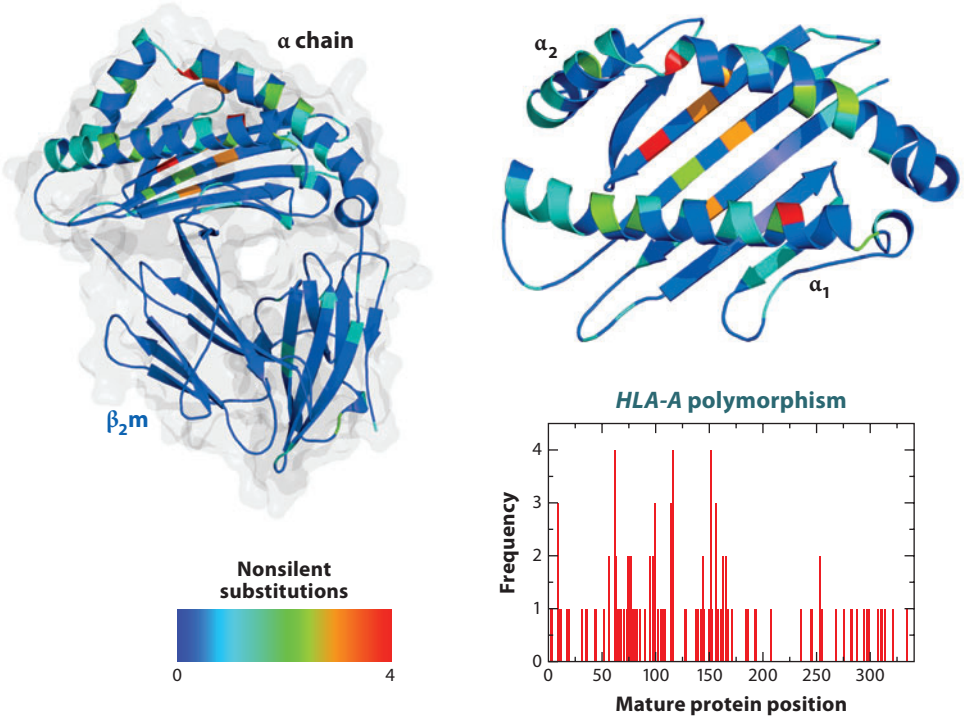


Figure 1

Major histocompatibility complex class I polymorphism. Structural and graphical representations of nonsilent polymorphism in the *HLA-A* gene. The frequencies of amino acid substitutions in eligible sequences of *HLA-A* (*A*01-A*03*, *A*11*, *A*23*, *A*24-A*26*, *A*29-A*34*, *A*36*, *A*43*, *A*66*, *A*68*, *A*69*, *A*74*, *and A*80*) were mapped onto the three-dimensional structure of HLA-A*02:01. Individual positions were color-coded according to the number of distinct amino acid substitutions observed: 0, blue; 1, cyan; 2, green; 3, orange; 4, red. Only complete and confirmed HLA-A sequences are represented.

EFFECTS OF HUMAN LEUKOCYTE ANTIGEN POLYMORPHISM ON ANTIGEN PRESENTATION

The classical *HLA* loci are the most highly polymorphic genes in the human genome, with the *HLA-B* locus possessing more than 1,000 alleles expressed as functional protein allotypes (20, 21). HLA allotypes may vary by more than 30 residues or as few as 1–2 residues (micropolymorphisms). Allotype differences predominantly map to the residues that contact a peptide within the peptide-binding groove (22), affecting its structural and electrochemical landscape and altering the environment within the peptide-binding pockets (see **Figure 1** and **Supplemental Figure 2**). Although all HLA molecules can bind a large range of peptides, changes within this landscape alter the amino acid side chains that can be accommodated within the peptide-binding pockets, hence constraining the peptide specificity of each polymorphic allotype. Different allotypes therefore possess different peptide-binding repertoires (the arrays of peptides that they bind) and different peptide-binding motifs (the preferred anchor residues that occupy the binding pockets) (23). The interaction of the MHC peptide and specific TCR is based on complementarity in both the structures and electrostatic features of the interacting surfaces. HLA allotypes with low similarity to one another have divergent contact sites for the TCR owing to changes in the exposed surface of the MHC and dissimilarity of peptide repertoires. HLA allotypes with differences of just one or

Supplemental Material

two amino acids (micropolymorphism) can alter the peptide repertoire, but often with substantial overlap (24, 25). However, even overlapping peptide repertoires expressed by similar but nonidentical HLA allotypes can potentially change the manner in which the same peptides are displayed, impacting T cell recognition and recruitment (15, 26, 27). Furthermore, micropolymorphisms can also alter the interaction of MHCI molecules with elements of the peptide-loading machinery. This alteration results in differing dependencies on the chaperone tapasin for peptide loading and surface expression and affects the thermostability of the final MHC-peptide complex, further impacting the peptide repertoire displayed (28–30). Buried cleft polymorphisms can also impact the conformation of the MHC (31) and the plasticity of the peptide (32, 33). Thus, structural variations in the MHC peptide presented to the TCR are likely to alter the T cell populations activated by the MHC (24, 26, 27).

T cell–mediated responses to infection depend on an individual's particular MHC allotypes' ability to load and present novel peptides from pathogens. HLA polymorphism is maintained because of the advantages of increased overall peptide repertoire provided by heterozygosity at the *HLA* loci, which in turn increases the chance of presenting peptides derived from a given pathogen. Thus, long-term progression to AIDS in human immunodeficiency virus (HIV) infection is slower in HLA heterozygotes than it is in homozygotes (34). Nonetheless, surprisingly few associations among particular HLA alleles and protective immunity phenotypes have been identified, yet a growing number of ADRs have been associated with specific HLA allotypes (summarized in **Table 1**). Although the distinct pathologies of each ADR challenges the idea of a common mechanism, clinical diversity in outcome could also reflect differences in drug metabolism, MHCI versus MHCII function, and the tissue specificity of peptides involved in the generation of the immunogenic complexes.

RECOGNITION OF SMALL MOLECULES BY T CELLS

Studies of DTH led Pichler and associates (6) to propose two mechanisms for MHC-dependent T cell stimulation by different drugs (**Figure 2**). The first is the hapten/prohapten concept, and the second is the concept of pharmacological interaction with immune receptors (the p.i. concept). The hapten/prohapten concept proposes that the drug or a reactive metabolite (hapten/prohapten) reacts with a self-protein or peptide to generate a novel, haptenated product. This product then undergoes antigen processing to generate a novel MHC ligand that is loaded onto the MHC and trafficked to the cell surface, where it activates antigen-specific T cells (6, 35). Presentation of drug-modified MHC-peptide complexes is presumed to require metabolically active, antigen-processing competent cells for the generation of the immunogenic complex. In addition, a lag period between drug loading of the APCs and the presence of the immunogenic MHC-peptide at the cell surface is also observed, consistent with the time taken for metabolism and antigen processing to produce a sufficient amount of novel MHC-peptide to stimulate a T cell response. Once generated, these altered MHC-peptide complexes are as stable as conventional MHC-peptide complexes, and ligand removal requires peptide exchange or peptide stripping from the groove. Examples of T cell responses induced by drug-modified peptides include responses to penicilloyl peptides in the presence of penicillins (36, 37) and responses to nitroso-sulfamethoxazole-modified peptides during sulfamethoxazole treatment (38).

In cases in which drug sensitization of target cells for T cell recognition is fast, the immunogenic complexes produced by drug introduction are unlikely to depend on antigen processing or cellular metabolism. This prompted the proposal of the second concept for MHC-dependent T cell stimulation by different drugs: the concept of pharmacological interaction with immune receptors, or p.i. concept. This model involves a noncovalent, labile interaction of the drug with the MHC at

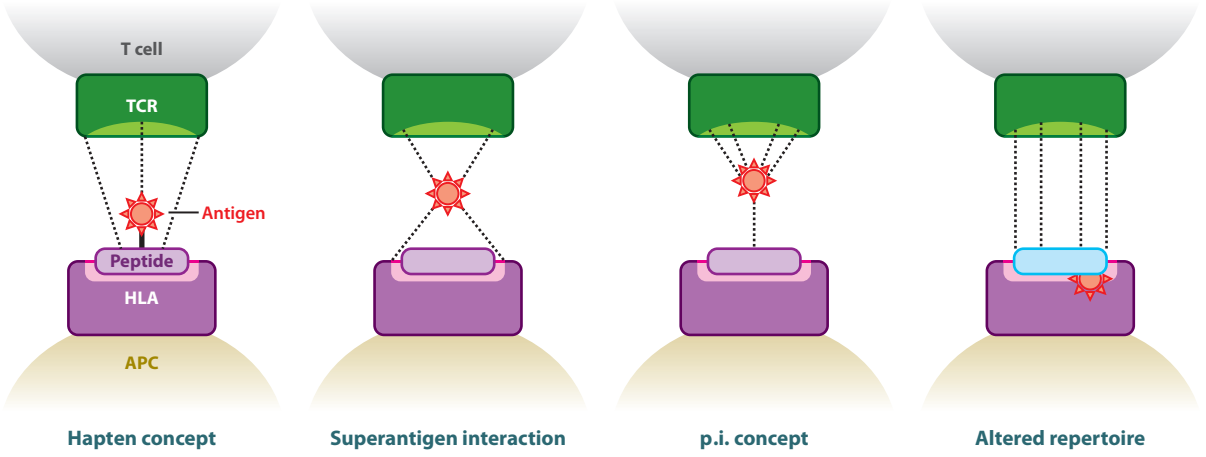


Figure 2

Mechanisms of T cell stimulation by small-molecule antigens. Schematic representation of the different modes of interaction of metals/drugs with T cell receptors (TCRs) and major histocompatibility complexes (MHCs), of which the hapten concept and the concept of pharmacological interaction with immune receptors (p.i. concept) represent the two extremes. A novel concept is anchor site occupation by small-molecule antigens, which induces an altered peptide repertoire, whereas superantigens are proposed to directly link T cell receptors and MHCs in a peptide-independent manner. Solid lines represent covalent interactions; dashed lines represent noncovalent interactions. Adapted from Reference 39. Other abbreviations: APC, antigen-presenting cell; HLA, human leukocyte antigen.

the cell surface. Such an interaction requires neither metabolism nor antigen processing, resulting in the near immediate generation of the immunogenic complex. Furthermore, the p.i. concept also explains how immunogenic complexes may be formed on introduction of the drug to aldehyde-fixed, thus metabolically incompetent, cells. It is also observed in these cases that simple washing of the cells and removal of the drug from the cell media is sufficient to abrogate APC-mediated stimulation of drug-specific T cells, supporting a much more unstable ligand than a haptenated peptide (39). Drug-specific T cell responses generated via this pathway are suggested for lidocaine, lamotrigine, and sulfamethoxazole in its nonreactive form (40–42).

POTENTIAL MECHANISMS UNDERPINNING HUMAN LEUKOCYTE ANTIGEN CLASS I–ASSOCIATED DRUG HYPERSENSITIVITIES

The strongest of HLA associations are *HLA-B*57:01* with abacavir hypersensitivity syndrome (AHS) in the Caucasian population (43–45), *HLA-B*15:02* with carbamazepine-induced Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Asian populations (46–48), and *HLA-B*5801* with allopurinol hypersensitivity syndrome (HSS) and SJS/TEN (49–51), with odds ratios greater than 500, 1,000, and 800, respectively. Each of these diseases is associated with a particular HLA class I allotype, displays clear T cell involvement, and develops along a timeline consistent with induction of T cell–mediated responses (43, 52–56). Although all of the HLA associations, weak or strong, may involve direct mechanistic roles for the HLA allotypes in disease progression, weaker associations may indicate additional contributing factors that complicate the analysis of fundamental mechanisms of drug hypersensitivity. The strengths of the AHS associations, carbamazepine-induced SJS/TEN associations, and allopurinol HSS associations, as well as clear evidence for T cell involvement in each of these hypersensitivities, suggest simpler avenues for interrogation of the relationships between the HLA allotypes and T cell stimulation.

AHS: abacavir hypersensitivity syndrome

SJS: Stevens-Johnson syndrome

TEN: toxic epidermal necrolysis

HSS: hypersensitivity syndrome

In the case of AHS and *HLA-B*57:01*, in vitro studies have shown that drug-specific $CD8^+$ T cell responses can be elicited in response to abacavir-treated APCs possessing the HLA-B*57:01 molecule but not in response to APCs expressing closely related allotypes (57). However, there has been limited study into the roles of HLA allotypes in other drug hypersensitivity associations. Previous work on AHS provides strong functional evidence for a direct role of the HLA-B*57:01 molecule to explain the association of *HLA-B*57:01* with AHS. In the following section, we use this example as a mechanistic model to illustrate the activation of T cells by HLA class I–restricted presentation of drugs or their metabolites.

HLA-B*57:01 AND ABACAVIR HYPERSENSITIVITY SYNDROME: A NEW VARIATION OF THE HAPTEN/PROHAPTEN MODEL

Abacavir is an antiretroviral used in the treatment of human immunodeficiency virus type I (HIV-I) infection. Before the discovery of its association with *HLA-B*57:01* and the introduction of prescreening of patients for this allele, AHS occurred in approximately 5% of individuals treated with abacavir. AHS is clinically characterized by systemic symptoms including fever, rash, nausea, vomiting, abdominal pain, lethargy, and malaise occurring within 6 weeks of drug administration (median onset of 11 days). Cessation of abacavir treatment resolves this reaction, whereas reintroduction results in a more severe response and even death (8, 58). $CD8^+$ T cells are implicated in disease progression owing to the presence of $CD8^+$ T cell infiltrate in AHS-associated rash and patch testing (52). Furthermore, in vitro studies have demonstrated that $CD8^+$ T cell depletion leads to the abrogation of abacavir-associated increases in extracellular tumor necrosis factor-α (TNF-α) in AHS patients (43). The association of AHS with the *HLA class I* allele *HLA-B*57:01* was identified in 2002 by two independent laboratories (44, 45). Initially, the strength of the association was clouded by false-positive diagnosis of AHS, but the introduction of immunological confirmation via patch testing allowed the PREDICT-1 clinical trial and the SHAPE study to clearly determine a positive predictive value of 47.9% and a negative predictive value of 100% of *HLA-B*57:01* for AHS (52, 59). Initial reports that AHS is also linked to a haplotypic Hsp70-Hom M493T variant (43) have not been confirmed (D. Nolan, E. Phillips & S. Mallal, personal communication). Preclusion from abacavir treatment on the basis of screening for *HLA-B*57:01* has reduced the incidence of AHS and has decreased the unnecessary withdrawal of abacavir treatment in suspected AHS cases (59–61). As a result, screening for *HLA-B*57:01* is recommended by the FDA before the initiation of abacavir use in HIV-I treatment (62).

In 2008, we published data from an in vitro study of abacavir-induced T cell activation (57). The data demonstrated that the ability of abacavir-treated cells to stimulate T cells could be completely abrogated by a single amino acid residue change at position 116 (Ser116Tyr), which lines an anchor pocket in the peptide-binding groove of the HLA-B*57:01 molecule. This study also showed that the generation of the immunogenic complex was kinetically consistent with antigen processing, that it depended on TAP and tapasin, and that presentation could not be elicited by a range of HLA allotypes closely related to HLA-B*57:01. We have since observed that responsive T cell populations contain multiple TCR clonotypes, so there is no clear shared TCR variable beta gene (Vβ) usage among individuals (P. Illing, L. Kostenko, M. Bharadwaj & J. McCluskey, unpublished data).

This gives rise to two major questions: How is the ligand generated, and how is its presentation restricted to the HLA-B*57:01 molecule? Abacavir is a prodrug that, in order to mediate its antiviral effects, must first undergo deamination and phosphorylation to generate the guanosine triphosphate analog carbovir triphosphate that is responsible for inhibition of viral replication (63, 64). An attractive possibility is that the reactive metabolites formed during this process might

modify a limited set of cellular proteins, potentially the enzymes or chaperones involved in this process such as alcohol dehydrogenase (65). The degradation products of these putative drug-modified proteins might then furnish a novel immunogenic peptide, or peptide-drug complex, thus explaining the apparent necessity for antigen processing (**Figure 3*a***).

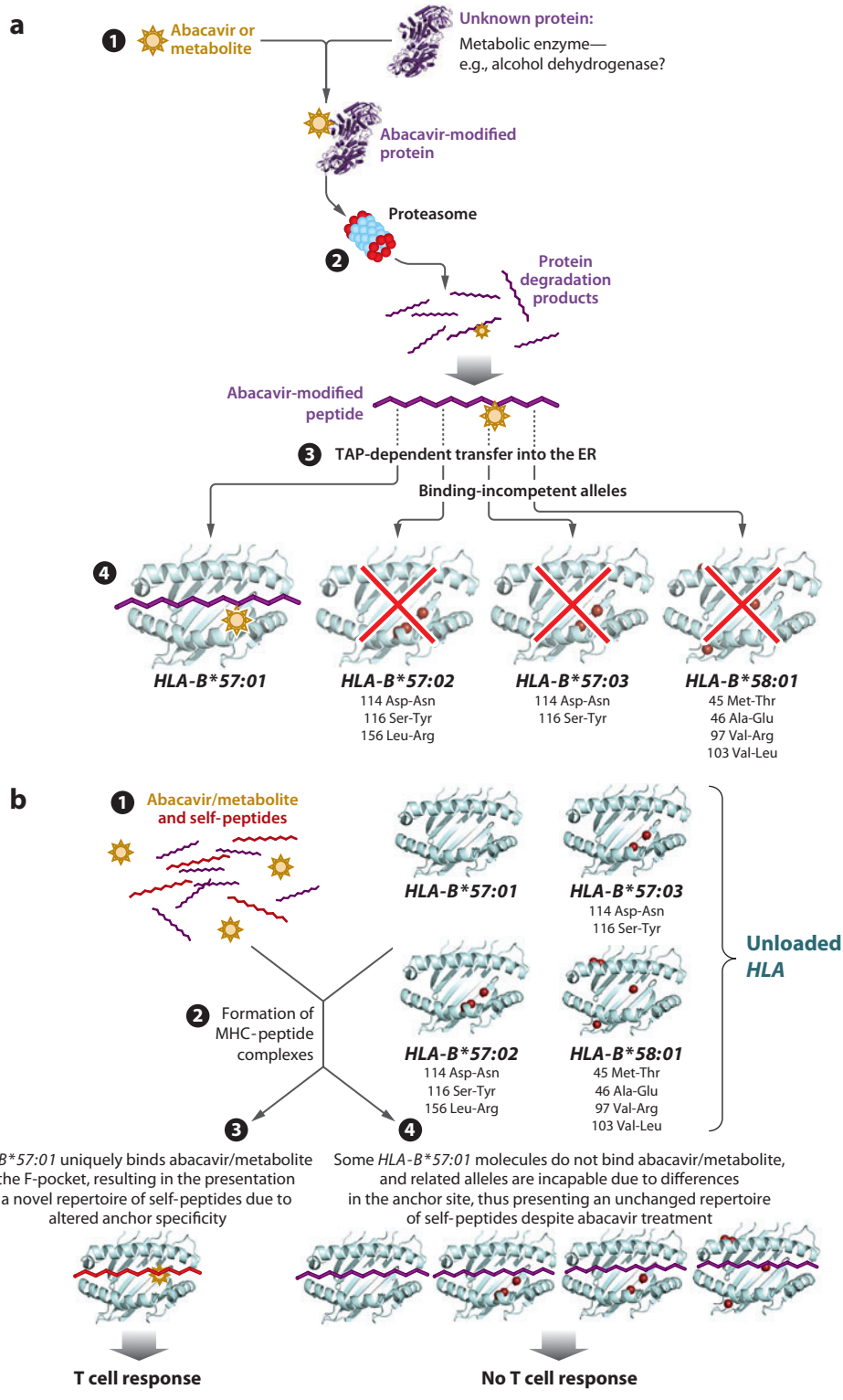
Our observation that abacavir-specific T cell responses can be induced in healthy (i.e., HIV-negative) HLA-B*57:01-positive blood donors in vitro (57) suggests that the ADR is unrelated to HIV infection and that the putative haptenated self-ligand is endogenously available in autologous peripheral blood mononuclear cells or in the culture medium. This conclusion raises the possibility that serum or APC-derived antigens provide the immunogenic carrier molecule.

Alternatively, the necessity for antigen processing could also be explained by incorporation of abacavir, or a metabolite, into an anchor pocket of the peptide-binding groove of HLA-B*57:01 molecule during folding within the ER. Incorporation in this manner would lead to a change in the landscape of the groove and thus a change in the peptide repertoire that is capable of binding; these changes could result in a large number of previously unseen peptides presented at the cell surface, eliciting a response akin to alloreactivity (**Figure 3*b***).

Either way, the abrogation of stimulation of drug-responsive T cells by the 116 Ser-Tyr mutation suggests that this area of the peptide-binding groove is key in the presentation of the immunogenic ligand or ligands and in their restriction to HLA-B*57:01. Together, these observations suggest several possible ways that the generation of an immunogenic complex could be restricted to a specific allotype. First, a drug-induced modification of a haptenated peptide occupies an anchor pocket in the HLA molecule and can be accommodated only by that allotype. Second, the drug or a metabolite is incorporated into a uniquely shaped anchor pocket of the associated allotype, altering its peptide-binding groove and generating a shift in the peptide repertoire able to bind. Third, a peptide that uniquely binds a particular HLA allotype is modified at one of its solvent-exposed side chains. Fourth, a drug-modified peptide acts as a mimotope for a pathogen-derived peptide against which the individual has previously mounted an immune response in the context of the associated allotype. Although we have been able to expand abacavir-responsive $CD8^+$ T cells from the blood of HLA-B*57:01-positive, abacavir-naïve individuals, we have no evidence that these arise from a cross-reactive antiviral T memory population. Furthermore, as T cell expansion is much slower in cultures from naïve HLA-B*57:01-positive individuals

Figure 3

A model for restricted generation of immunogenic complexes in abacavir hypersensitivity syndrome (AHS). (*a*) The hapten/prohapten model. ❶ Abacavir, or a metabolite thereof, modifies a cellular protein during its metabolism by the cell. Modification may be restricted to a few proteins by virtue of a fleetingly present reactive metabolite that rarely exits the active site of a metabolizing enzyme and thus has limited opportunity to haptenate proteins other than the responsible enzyme. ❷ The modified protein undergoes proteasome-mediated degradation to produce peptide fragments, including a drug-haptenated peptide. ❸ This peptide is transferred into the endoplasmic reticulum (ER) and loaded onto HLA-B*57:01 in a transporter associated with antigen processing (TAP)- and tapasin-dependent manner. This process is time dependent, and a delay is seen between drug administration and the presence of the immunogenic ligand at the cell surface. ❹ Micropolymorphisms within the antigen-binding groove between HLA-B*57:01 and related alleles (*highlighted by red balls on the ribbon structures of the antigen-binding clefts*) prevent loading of this peptide or ligand. In the context of HLA-B*57:01, this neoantigen stimulates antigen-specific $CD8^+$ T cells via T cell receptor (TCR) interaction. The neoantigen is bound to HLA-B*57:01 with stability similar to that of conventional human leukocyte antigen (HLA) ligands, and, once formed, it remains present on removal of abacavir from the culture medium. (*b*) Anchor site modification/occupation model. Abacavir, or a metabolite, specifically occupies the F-pocket of HLA-B*57:01, resulting in the presentation of a novel repertoire of self-peptides at the cell surface.



compared with cultures from AHS patients, we propose that this results from expansion from the naïve repertoire in naïve individuals. Thus, we do not consider that heterologous immunity due to prior viral exposure is responsible for the T cell reactivity in AHS. Identifying the ligand(s) responsible for AHS and determining its structure in association with HLA-B*57:01 are important in illuminating mechanisms further.

T CELL ACTIVATION VIA THE P.I. CONCEPT AND RESTRICTION TO SPECIFIC HUMAN LEUKOCYTE ANTIGENS

Whereas AHS remains the best understood of the drug hypersensitivities requiring classical antigen presentation for T cell stimulation, in vitro studies into T cell activation in carbamazepine hypersensitivities seem to indicate an alternate pathway. Carbamazepine is an anticonvulsant used in the treatment of epilepsy and bipolar disorder; however, owing to its mood-stabilizing effects, it also is used in the treatment of attention deficit hyperactivity disorder, trigeminal neuralgia, and schizophrenia. Carbamazepine causes use- and voltage-dependent stabilization of the voltage-dependent sodium channels of neurons to mediate these effects (66, 67).

Carbamazepine causes several DTH reactions including maculopapular exanthema (MPE), SJS/TEN, and HSS. An association between *HLA-B*15:02* and carbamazepine-induced SJS/TEN was observed in the Han Chinese population (47). *HLA-B*15:02* was present in 100% of affected individuals, but only in 3% of carbamazepine-tolerant individuals and 8.6% of the general population (47). Studies in both Indian and Thai populations have since found a similar association (46, 68). No such association is observed in Caucasian or Japanese populations, which have a lower incidence of *HLA-B*15:02* (69–71), nor does the association extend to carbamazepine-induced MPE or HSS (48).

SJS and TEN are considered different parts of the same disease spectrum, with mortality rates of 5% and 30%, respectively (72). They are severe bullous skin diseases characterized by bullae resulting from epithelial cell apoptosis on epidermal and mucosal surfaces and are classified on the basis of the extent of epidermal detachment. Less than 10%, 10%–30%, and greater than 30% detachment are classified as SJS, SJS/TEN overlap, and TEN, respectively (51). A $CD3^+$ T cell infiltrate, dominated by the $CD8^+$ T cell subset, is found within the lesions, and keratinocyte cytotoxicity is thought to be mediated by an MHCI pathway (53–56). Consistent with their nature as DTH-like responses, carbamazepine-induced SJS/TEN occurs within 2 months of drug administration, with a median onset of 15 days (48).

In vitro studies of activation of T cells in carbamazepine hypersensitivities have shown that carbamazepine-specific T cells can be expanded from patient blood many years after resolution of clinical symptoms. Furthermore, both $CD4^+$ and $CD8^+$ T cells can be reactivated in vitro in response to carbamazepine and its various metabolites in the absence of antigen processing (72–75), and recent reports suggest that a restricted TCR usage is crucial for carbamazepine-induced SJS (76). It has also been reported that sequencing of peptides from carbamazepine-treated *HLA-B*15:02* cells did not reveal carbamazepine-modified peptides, although natural peptides were identified and unaltered carbamazepine was also detected (77).

There is also some evidence that *HLA-B*15:02* is associated with SJS/TEN in response to another anticonvulsant, phenytoin, which binds the same site on the voltage-dependent sodium channels as the site bound by carbamazepine (46, 78). The suggestion that both anticonvulsants bind the sodium channel in a similar manner suggests that they may also interact with HLA-B*15:02 in a similar fashion, but the differences in their structures may elicit stimulation of differing T cell clones, which would explain a lack of cross-reactivity among drug-specific T cells (75).

Although far from conclusive, these data seem to indicate T cell stimulation following the p.i. concept rather than via a haptenated peptide. In such a case, the restriction of presentation to a specific allotype implies a specific interaction that has sufficient stability with both the drug and the TCRs of reactive T cells to induce a T cell response. As the interaction appears to occur immediately at the cell surface, it is unlikely that the drug is occupying any of the peptide anchor sites or interacting during peptide loading to allow the binding of peptides outside the normal repertoire. Therefore, it seems that the peptide interacts with intact MHC-peptide complexes. In this model, it is unclear whether a specific peptide or many MHC-peptide complexes allow stable interaction of the drug. Although a monoclonal or pauciclonal T cell response may indicate the recognition of a single MHC-peptide-drug complex, a polyclonal T cell response does not necessarily indicate a multitude of permissive peptides. In any event, the isolation of peptides involved in p.i. concept hypersensitivity may present a much greater challenge than the isolation of haptenated peptide ligands because there is no covalent labeling of the peptide by the drug to aid identification.

Confounding the idea that carbamazepine can interact only with the HLA-B*15:02 molecule is the T cell activation evident in MPE and HSS, which are not associated with HLA-B*15:02. Clear delineation of these diseases allowed investigators to find the strength of the association between carbamazepine-induced SJS/TEN and HLA-B*15:02 (48). However, a recent study into DRESS (another term for DHS) in response to carbamazepine, allopurinol, and several other drugs has suggested that the reactivation of Epstein-Barr virus infection is responsible for T cell activation and proliferation (79). Such a scenario makes it more plausible to contemplate the cross-reactivity of drug-induced T cells among nonstructurally related drugs that have similar pharmacological effect (e.g., carbamazepine and valproic acid). Whether drug presentation or virus reactivation could explain the differences in these hypersensitivities and the observed associations requires further study.

MECHANISMS UNDERLYING HUMAN LEUKOCYTE ANTIGEN CLASS II–ASSOCIATED DRUG HYPERSENSITIVITIES

The HLA class I–associated drug hypersensitivity reactions are better defined mechanistically compared with the HLA class II–restricted DHS, which largely remains unexplored. The most common drug reactions associated with HLA class II allotypes involve the nonsteroidal anti-inflammatory drugs (NSAIDs), which encompass a range of hypersensitivity reactions. In fact, aspirin (acetylsalicylic acid), one of the most widely used NSAIDs in medical practice, causes two phenotypically distinct hypersensitivity reactions: aspirin-induced urticaria, associated with the *HLA-DRB1*13:02–DQB1*06:09* haplotype (80), and aspirin-intolerant asthma, associated with *HLA-DPB1*03:01* (81, 82). In addition, some of the common hypersensitivities triggered by food/environmental agents such as nickel (Ni), beryllium (Be), and wheat gluten are HLA class II linked and have been under investigation for more than two decades. Although these agents are not strictly therapeutic pharmaceuticals, they are relevant to understanding the mechanistic models of the immune response in HLA class II–associated hypersensitivity reactions.

Chronic beryllium disease (CBD), also known as berylliosis, is a granulomatous disorder that primarily affects the lungs and lymphatics (83) and is characterized by the accumulation of Be-responsive $CD4^+$ T cells. CBD results from occupational exposure to Be, and genetic susceptibility to CBD has been strongly associated with the MHCII isotype HLA-DP, especially HLA-DPB1*02:01 and other allotypes that contain a glutamic acid residue at position 69 of the β chain (βGlu69) (84–88). On the basis of their genetic dispositions and the type of exposure, up to 20% of workers exposed to Be can acquire CBD. Thus, with approximately 1 million individuals exposed

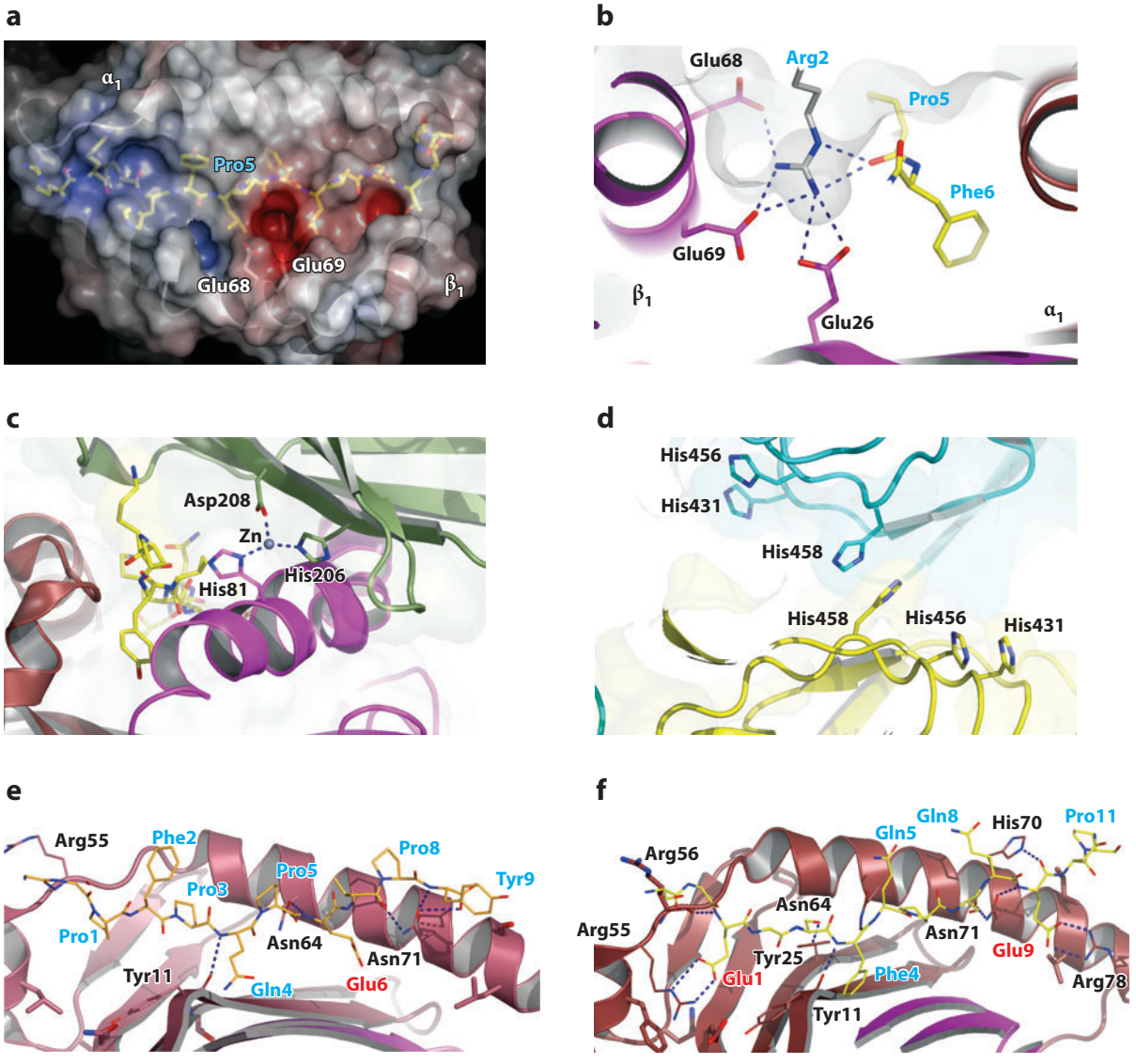
to Be (89) in nuclear reactors and in the mining, ceramic, aviation, and jewelry industries, CBD is a serious public health concern. $CD4^+$ T cells play a critical role in the immunopathogenesis of CBD, and a large frequency of Be-specific $CD4^+$ T cells (mean of 18%) have been documented in the bronchoalveolar lavage of CBD patients (90). Previous studies have shown that soluble HLA-DP molecules expressing βGlu69, but not HLA-DP molecules with lysine residues at this position, can bind Be in vitro with high affinity (91), raising the possibility that Be could bind directly to HLA-DPβGlu69 molecules. This hypothesis has been further strengthened by recent observations arising from the crystal structure of HLA-DP2 bound to a self-peptide derived from the HLA-DR α chain (92). This study revealed that the peptide-binding groove between the peptide backbone and the HLA-DP2 β-chain α helix contains a gap that exposes βGlu69 as part of an acidic pocket that could easily accommodate a compound the size of a Be-containing complex; this finding resonates with the p.i. concept of drug interaction with the MHC (**Figure 4*a*,*b***). Mutation of βGlu69—or mutation of either of the two other glutamic acid residues in this pocket, βGlu26 and βGlu68—abolished Be presentation. Perplexingly, 15% of CBD patients do not possess a βGlu69-containing HLA-DP allotype; instead, an increased frequency of HLA-DR13 alleles that possess an acidic cluster composed of βAsp28, βAsp70, and βGlu71 is observed in such patients (84, 87). These observations suggest that other MHCII allotypes may also be associated in Be-induced disease, and further studies should be conducted to confirm these associations.

Ni is another of the key metals associated with contact allergy: Up to 30% of the population is affected (93), largely owing to allergic skin reactions to Ni-containing jewelry (rings and earrings) and more recently to cellular telephones (94, 95). As Ni is also commonly used for cardiovascular stents and orthopedic and dental implants, such reactions are serious and have important implications for biocompatibility. The relevance of Ni allergy in the failure of orthopedic implants and cardiac devices is, however, still unclear. Previous work has shown that Ni is a hapten that binds to proteins and induces DTH, which is clinically detected via a patch test. Ni has been the most frequent allergen detected in patients patch-tested for allergic contact dermatitis worldwide

Figure 4

Structural insights into Be, Ni and gluten hypersensitivity. (*a*) Representation of the HLA-DP2 antigen binding cleft viewed from above, with the HLA-DRA-derived self-peptide shown in stick format (*yellow*). The molecular surface of the MHC-peptide complex has been overlaid in semitransparent mode, and the ±10 kT/e electrostatic potential from the solvent-accessible surface is rendered on the molecular surface. The exposed acidic pocket is formed by three residues of HLA-DPB1*02:01: Glu26, Glu68, and Glu69. (*b*) The network of ionic and hydrogen-bonding interactions formed between residues lining the exposed acidic pocket of HLA-DP2 and the embedded guanidinium group of a symmetry-related arginine residue, observed in the crystal structure. The molecular surface of HLA-DP2 is overlaid in semitransparent mode. (*c*) The crystal structure of HLA-DR1 in complex with the Zn-dependent bacterial superantigen, *Staphylococcus aureus* enterotoxin H (1HXY; see Reference 142). His81 on the HLA-DR1 β chain is involved in Zn coordination, along with the superantigen residues His206 and Asp208, in a manner that may resemble that of Ni-mediated interactions of DR1 with selected TCRs. For example, His81 is essential for Ni-mediated activation of the HLA-DR-promiscuous human T cell clone, SE9 (102). (*d*) Cluster of six His residues observed at the interface of the human TLR4 homodimer, in its complex structure with MD2 (3FXI; see Reference 103). Mutagenesis studies have shown that these residues are essential for Ni-mediated proinflammatory activation of human TLR4. In the presence of Ni, the His side chains are predicted to reorient themselves, thereby forming a pair of metal-binding sites that result in the cross-linking of TLR4 monomers into a dimer that structurally resembles the signaling form of the receptor. (*e*) Representation of the α_1 domain region of the antigen-binding cleft of HLA-DQ2 (1S9V; see Reference 108). The α_1-gliadin peptide $Q_{-3}LQPFPQPELPY_9$ and selected residues of HLA-DQ2-α appear in stick format. Key hydrogen-bond interactions between HLA and peptide are shown as blue dashes. Residues within the antigen-binding cleft that are not conserved between HLA-DQ2 and HLA-DQ8 appear as thicker sticks. Peptide residues are labeled in light blue, and deamidated positions are in red. (*f*) Same view as panel *e* of HLA-DQ8 in complex with the α_1-gliadin peptide $Q_{-6}QYPSGEGSFQPSQENPQ_{12}$ (2NNA; see Reference 111). Abbreviations: Arg, arginine; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Glu, glutamic acid; His, histidine; HLA, human leukocyte antigen; MHC, major histocompatibility complex; Ni, nickel; Phe, phenylalanine; Pro, proline; TCR, T cell receptor; TLR, Toll-like receptor; Tyr, tyrosine; Zn, zinc.

(95, 96), and owing to its significant public health importance (97), Ni was named the Contact Allergen of the Year in 2008 by the American Contact Dermatitis Society. European nations have implemented legislation to limit the release of Ni from objects intended to be in prolonged contact with the skin (98). Ni is a potent stimulator of cellular activation, capable of inducing signal transduction in both dendritic cells and endothelial cells (99). HLA-restricted αβT cells with specificity for Ni ions were isolated from the blood and skin lesions of Ni-allergic patients more than a decade ago (100, 101). Subsequent studies based on a Ni-specific T cell clone revealed the potential contact sites of interaction between the TCR and the restricting HLA-DR β chain (102). It was established that Ni reactivity was neither dependent on protein processing in APCs nor affected by the nature of HLA-DR-associated peptides. The T cell activation by Ni was shown to depend on Tyr29 in CDR1α, an *N*-nucleotide-encoded Tyr94 in CDR3α, and a conserved His81 in the HLA-DR β chain. This suggests that, like superantigens (**Figure 4*c***), Ni may directly link



TCRs and MHCs in a peptide-independent manner; however, unlike superantigens, Ni requires CDR3α-determined TCR amino acids. Apart from the antigen-specific T cell–activating signal, a second proinflammatory stimulus is also required for efficient Ni sensitization, and, until recently, the specific proinflammatory receptor and the mechanism of its activation remained largely elusive. In a landmark study by Schmidt et al. (103), a specific region of human Toll-like receptor 4 (TLR4) (amino acids 369–616) was identified as the Ni receptor, leading to the modeling of the specific interaction of the imidazole groups of the histidines in this region (His456 and His458) with the Ni^{2+} ions (**Figure 4*d***). This model illustrated that Ni is capable of activating TLR4 homodimer formation, leading to signal transduction and production of proinflammatory cytokines, and that Ni^{2+}-induced proinflammatory responses are species specific and require nonconserved histidines that are found in human TLR4 but not mouse TLR4.

Celiac disease is one of the most common inflammatory disorders of the small intestine (104), triggered by cereal proteins such as wheat gluten and related proteins in barley and rye. The onset of celiac disease generally occurs in childhood, but adults can also develop it (105). Clinical manifestations vary according to age group: Young children usually present with abdominal symptoms (diarrhea, abdominal distension), whereas in adults, gastric symptoms can be accompanied by silent manifestations such as anemia, osteoporosis, or neurological symptoms (104). Celiac disease has been strongly associated with HLA-DQ2 and/or HLA-DQ8 (106) and is clinically diagnosed on the basis of intestinal biopsy, the detection of circulating autoantibodies to the enzyme tissue transglutaminase, and supporting evidence of genetic predisposition. Deamidated gluten peptides are thought to be presented to $CD4^+$ T cells in the context of HLA-DQ2 or HLA-DQ8 molecules that are on the surfaces of APCs (**Figure 4*e,f***)—mainly macrophages, dendritic cells, and B cells. HLA-DQ2-restricted peptides generally prefer glutamate at anchor positions P4 or P6, and sometimes P7. Antigen presentation studies and epitope mapping of T cell responses in celiac disease suggest that the interaction between the wild-type dominant gluten peptide and HLA-DQ2 is of low affinity but that it can be enhanced by deamidation of P6 glutamine in particular (107). Structural analysis of HLA-DQ2 shows how a network of hydrogen bonds binds a dominant, deamidated gliadin determinant to HLA-DQ2 (108). This binding is thought to improve antigen presentation, thereby driving T cell recognition and intestinal disease in susceptible patients (107, 109). In contrast to the protease resistance of the dominant DQ2-restricted gliadin peptides that drive celiac disease, the dominant gliadin peptides appear to be protease sensitive in patients with DQ8-associated disease. The HLA-DQ2-associated and HLA-DQ8-associated forms of celiac disease have distinct patterns of deamidation dependency of the relevant gluten peptides that mediate T cell reactivity. The single deamidation step at P6 for HLA-DQ2-linked peptides contrasts with optimal deamidation steps at positions P1 and P9 for HLA-DQ8-associated celiac disease (110). Henderson et al. (111) showed that this dual deamidation of glutamates at P1 and P9 in gliadin peptides enabled buried salt-bridging interactions with HLA-DQ8. In both forms of disease, DQ2- and DQ8-associated $CD4^+$ T cells are activated and secrete inflammatory cytokines such as interferon-gamma (IFN-γ). This process can, in turn, boost HLA-DQ2/8 expression and induce the release of proteolytic matrix metalloproteinases by myofibroblasts, resulting in the villus atrophy and crypt hyperplasia characteristic of celiac disease. Other cytokines such as interleukin-18 (IL-18), IFN-α, and IL-21 also seem to play a role in polarizing and maintaining the inflammatory response. The tissue damage leads to the release of transglutaminase-gluten complexes, which results in the production of autoantibodies to both gluten and transglutaminase via determinant spreading. Although the immunopathology of celiac disease is well defined, the exact factors that trigger its development are still unclear.

MECHANISM(S) INVOLVED IN DRUGS ASSOCIATED WITH HUMAN LEUKOCYTE ANTIGEN–MEDIATED HEPATOTOXICITY

Common patterns of pathogenesis (systemic/localized) seen in different DHS reactions may reflect common mechanisms and/or sites of drug metabolism that trigger generic immunological events mediated by different *HLA* alleles. For example, hypersensitivity to both carbamazepine and allopurinol can lead to skin lesions and SJS/TEN, whereas hypersensitivity reactions to flucloxacillin and ximelagatran are associated with drug-induced liver injury (DILI)—a rare but serious reaction, considering that 75% of individuals with acute liver failure either die or require liver transplantation (112). It has been speculated that DILI is primarily triggered by the interaction of the drugs or their reactive metabolites with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and/or oxidative stress (113). Furthermore, the reactive intermediates may also cause mitochondrial dysfunction through the disruption of ionic gradients and intracellular calcium stores. The resulting impairment of cellular function can potentially lead to cell death and liver failure. A role for specific hepatic pathways in drug metabolism also seems likely. One of the strongest HLA associations with DILI is that of flucloxacillin and *HLA-B*57:01*, with an observed odds ratio of 80 for disease development (114). Despite the strong link, the mechanism for flucloxacillin DILI is poorly understood; there is some evidence of drug-specific T cell reactivity (115), implying that the HLA-associated presentation of the drug or its metabolite potentially induces an inflammatory T cell response that culminates in cellular damage and liver toxicity. The association of *HLA class II* genes or haplotypes with DILI is far more common, albeit not as strong as that seen with *HLA-B*57:01*. These associations include lapatinib (*HLA-DRB1*07:01-DQA1*02:02/DQB1*02:03*) (116), ximelagatran (*HLA-DRB1*07* and *HLA-DQA1*02*) (117), and the antituberculosis drugs isoniazid (*HLA-DRB1*03*), rifampin (*HLA-DQA1*01:02*), and ethambutol (*HLA-DQB1*02:01*) (118). In a recent review, Russmann et al. (119) suggested a three-step working model wherein the parent drug or its reactive metabolite causes direct cell stress, mitochondrial inhibition, or specific immune reactions after MHCII-associated presentation of haptenated peptides to T cells, leading to a mitochondrial permeability transition that culminates in apoptosis or necrosis. Further studies are required to confirm the hypothesis and the importance of liver-specific metabolic pathways in the generation of novel ligands.

APPROACHES TO DEFINE DRUG CONJUGATES IN DRUG HYPERSENSITIVITY SYNDROME

As described above, drug interactions with HLA-peptide complexes can induce aberrant immune responses by generating novel T cell ligands for which immune tolerance is lacking. Methods to reliably detect and characterize such interactions are predominantly restricted to covalent conjugates, as these interactions will survive biochemical isolation and characterization of the active species. The isolation and characterization of HLA-bound peptides have advanced rapidly over the past decade (120), either through the mapping of determinants using synthetic peptides or through de novo analysis of peptide sequences using tandem mass spectrometry. In the case of drug conjugates, the synthetic peptide approach is limited because of the unknown identity of the modified peptide(s) and the lack of knowledge about the conjugation chemistry. Thus, the de novo sequencing of peptide-drug conjugates by mass spectrometry is preferable.

In this approach, cell-surface HLA-peptide complexes are typically isolated using affinity chromatography via immobilized monoclonal antibodies specific for the HLA allotypes. Once captured, the HLA-peptide complexes are eluted in acid, in a process that also facilitates the dissociation of the peptide ligand from the HLA proteins. The peptides are then separated from the proteins

through either chromatography or ultracentrifugation prior to analysis by liquid chromatography–tandem mass spectrometry.

Several approaches have been used to isolate naturally processed and presented HLA-bound peptides directly from cells; these include analysis of peptides contained within cell lysates (121), isolation of peptides directly from the cell surface (122, 123), and immunoaffinity purification of the MHC-peptide complexes from detergent solubilized cell lysates (23, 124–127). The simplest approach involves the extraction of peptides from whole cell lysates following treatment with an aqueous acid solution such as 1% trifluoroacetic acid. Typically, these preparations are fractionated by reversed-phase high-performance liquid chromatography and screened with a functional assay to confirm the presence of a particular T cell epitope. Titration of fractions into functional assays allows relative quantitation of known T cell epitopes extracted from the surface of different cell types (23, 128–130). In some circumstances, the peptides are amenable to sequencing of individual components of the fractionated material by mass spectrometry (125, 127).

An alternative to the acid lysis method utilizes a nonlytic approach for recovering cell-surface-associated peptides: incubation in an isotonic buffer that contains citrate at pH 3.3. This buffer facilitates dissociation of MHC-bound peptides from the cell surface without affecting cell viability (123), so that the same cells may be harvested daily in an iterative approach for obtaining MHC-bound material.

Although mass spectrometric approaches have been highly successful in identifying protein- and peptide-based allergens (131), no examples of identification of drug-modified HLA-peptide complexes have been reported in the literature. Perhaps the closest example is penicilloyl modification of peptides in penicillin-allergic individuals (132, 133): Investigators have used mass spectrometric interrogation of HLA-DR-bound peptides to define T cell responses to in vitro conjugated model peptides and modified naturally occurring ligands (133). The in vitro modification of these peptides was achieved through the conjugation of penicillin G to the epsilon amine of their lysine residues. Such HLA-DR-restricted responses do not require antigen processing (134), but naturally modified peptides from penicillin-treated cells or from patients' APCs have not been reported. Thus, the elaboration of naturally presented peptide-drug conjugates restricted by different HLA class I or class II molecules remains a future challenge.

MODULATING FACTORS AFFECTING PENETRANCE OF HUMAN LEUKOCYTE ANTIGEN RISK

Whereas HLA associations have been clearly established for abacavir, carbamazepine, and allopurinol, it is still not clear if genetic or environmental factors are associated with other DHSs such as those caused by sulfamethoxazole and nevirapine. Recent genomics technologies such as the genome-wide association studies facilitate an unbiased approach to study genetic risk in ADRs—specifically, thousands of *HLA* alleles of the extended human MHC can be studied. This approach can provide positive pharmacogenomic associations with fewer than 100 subjects (135). This technique was recently utilized in a DILIGEN study (114), which included 51 cases and 289 controls, to identify *HLA-B*57:01* as a potent risk factor for DILI from flucloxacillin. A similar approach identified a strong association of *HLA-DRB1*07* and *HLA-DQA1*02* with ximelagatran-induced elevated levels of serum alanine aminotransferase in a small case-control study (117).

The genome-wide analysis exploits the wisdom of systematic DNA banking during preclinical and clinical trials of new drugs. These DNA banks will ensure that retrospective genome-wide association studies can be efficiently performed to identify a predictive genetic marker if DHS does occur.

IMPLICATIONS FOR UNDERSTANDING HUMAN LEUKOCYTE ANTIGEN DISEASE ASSOCIATIONS

It remains intriguing that the relative risks (RRs) of the better-characterized HLA-linked ADRs are high (RR = 100–1,500) compared with the RRs for known immune phenotypes in protective immunity [e.g., long-term nonprogression associated with *HLA-B57* and *HLA-B27* in HIV (RR = 4–6)]. Given the central role and primary function of HLA in protective immunity, it is perhaps puzzling that there are not clearer-cut, stronger associations of particular HLA allotypes with immune protection or susceptibility phenotypes in infectious disease. The proposed mechanism for AHS suggests a possible explanation (**Figure 5**) for this apparent paradox. The evidence that a drug-mediated peptide modification might trigger an MHCI-dependent, $CD8^+$ T cell DTH reaction implies two layers of specificity in the process. First, the drug must interact with one or more host proteins to create a drug adduct or chemically modified polypeptide. The efficiency of adduct formation might be greatly enhanced if the drug were required to interact in a systematic manner with a restricted set of proteins such as chaperones or host enzymes for conversion of the prodrug to an active moiety. More stochastic interactions with serum or other cellular proteins might not produce any single species of modified peptide with any abundance. In contrast, stereotypic interaction with drug chaperones or modifying enzymes might produce significant quantities of identically modified polypeptides potentially available for further processing and TAP-dependent transport into the ER. A second layer of specificity resides in the constraints that HLA polymorphism imposes on the binding to MHCI molecules. MHC binding is determined by the chemical structure of the HLA cleft and is exquisitely specific for ligands with certain characteristics. The smaller the number of drug-modified self-peptides, the greater the probability that HLA-peptide binding will be restricted to a small number of HLA allotypes, perhaps even a single allotype, thereby creating a high RR for this allotype.

However, infection with complex pathogens generates a potentially large number of novel peptides that any HLA locus product can present to T cells. Thus, some degree of protective immunity is mounted even if not all HLA allotypes are contributing to T cell activation. In other words, HLA polymorphism and locus differences mask deficiencies in peptide presentation by specific allotypes when a large repertoire of novel peptides is available for binding during infection. The consequence of this HLA redundancy in peptide presentation is that specific HLA associations with protective phenotypes are rarely evident. Intriguingly, the strengths of HLA associations with autoimmune diseases such as type 1 diabetes and narcolepsy lie somewhere between those associations observed in protective immune phenotypes and those observed in ADRs. This might suggest that autoimmune disease susceptibility depends, initially at least, on T cell recognition of peptides from defined autoantigens rather than multiple self-targets.

FUTURE CHALLENGES

Clinical Implications

Researchers and pharmaceutical companies faced with well-defined ADRs, particularly those associated with small-molecule drugs and immunological in their manifestations, should consider HLA testing of their patient populations. HLA associations with ADRs are more likely to be detectable when the patient population is genetically homogeneous, the HLA typing is high resolution (4-digit, DNA-level), and the clinical ascertainment of a defined ADR is rigorous. Future management of HLA-associated drug hypersensitivities will involve cost-benefit decisions on pretreatment HLA screening and an evaluation of the mechanism underpinning DHS. It is possible

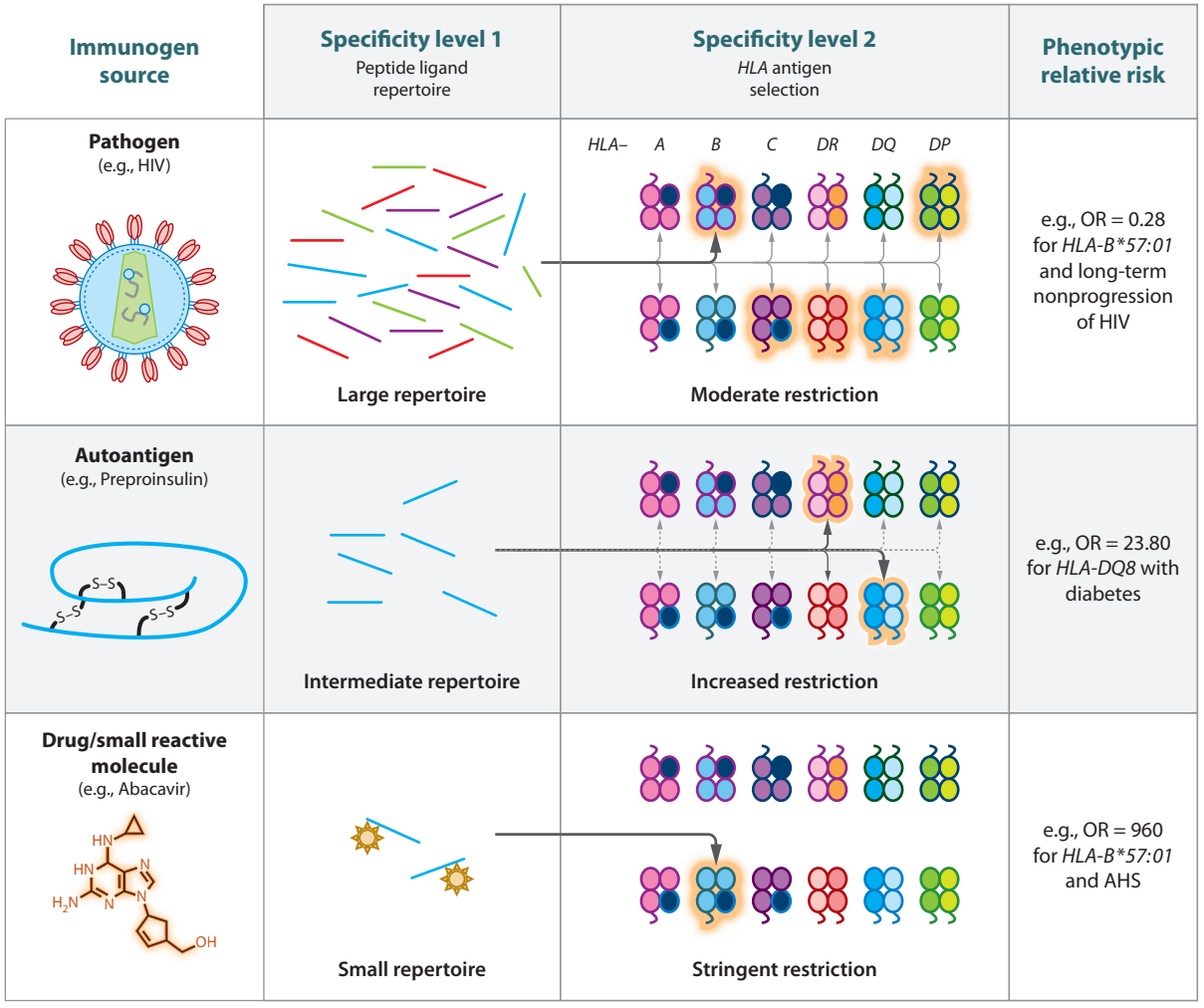


Figure 5

Conceptual model for the differences in relative risk of HLA-associated diseases. The differences in relative risk of HLA alleles for protective immunity, autoimmunity, and HLA-associated ADRs may arise from the two layers of specificity in T cell stimulation: the breadth of the potential repertoire of HLA ligands produced, and the HLA alleles capable of their presentation. Invading pathogens provide a relatively large pool of neopeptides derived from their protein components. Proteins and peptides involved in autoimmunity provide a relatively smaller peptide pool upon degradation, whereas drugs involved in HLA-associated ADRs may produce few haptenated ligands owing to the transient presence of a reactive metabolite capable of protein modification. As the peptide pool decreases, the stringency of HLA restriction increases. For example, whereas alleles of the HLA-B*57 family may be associated with protective immunity to HIV infection owing to increased proficiency in antigen presentation, the relative risk remains low owing to presentation of immunogenic ligands by other alleles. In contrast, the small pool of abacavir-modified peptides in AHS may restrict ligand presentation to HLA-B*57:01 only, resulting in a considerably higher relative risk of AHS. Overall, the model sustains the substantially higher odds for the association of specific HLA alleles with ADRs compared with those associated with autoimmunity and protective immunity. Abbreviations: ADR, adverse drug reaction; AHS, abacavir hypersensitivity syndrome; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; OR, odds ratio. References associated with data in right-most column (*top to bottom*): 136, 158, 43.

that in some cases, MHC-peptide reactive groups on new drugs can be removed or exchanged or that drug metabolic pathways that form immunological targets can be manipulated. For example, although Ni hypersensitivity is not HLA mediated, it is associated with TLR4 signaling through a Ni^{2+}-responsive region in human TLR4 that is distinct from the region's lipopolysaccharide-binding domain (103). This implies that it is possible to specifically interfere with Ni^{2+}-induced TLR4 signaling without affecting the cellular response to lipopolysaccharide. More studies of HLA-linked ADRs are required to determine whether these can be subverted either pharmacologically or by the development of alternative drug therapies for hypersensitive patients.

Human Leukocyte Antigen Screening

As the diagnosis of DHS is based primarily on clinical symptoms, a prerequisite to the determination of the HLA association is that the hypersensitivity has a clearly defined phenotype. However, a range of symptoms can be associated with adverse reactions to a single drug, suggesting different underlying mechanisms. For example, skin reactions to carbamazepine can range from mild MPE to more serious blistering skin rashes such as SJS/TEN. Unless uniform criteria are established for the diagnosis of specific hypersensitivity reactions, sorting out the genetic determinants of DHS reactions will be difficult (59). The European Registry of Severe Cutaneous Adverse Reactions recently developed criteria for the diagnosis of blistering reactions (137). There is urgent need for more such initiatives.

It is significant that the FDA recently recommended genetic testing before starting abacavir and carbamazepine therapies. However, HLA screening to prevent new ADRs should be implemented only after the veracity of the genetic association has been clearly established in a large population with a diverse ethnic background and after the cost-benefit ratio has been determined for testing in the target population. The association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN is one of the strongest associations of HLA-mediated DHS; however, it was largely derived from studies in Asian populations. A recent European study (71) of 12 carbamazepine-induced SJS/TEN cases (9 French and 3 German) found that the *HLA-B*15:02* allele is not a universal marker for this disease and that ethnicity matters. Among the 12 SJS/TEN cases, only 4 individuals (later determined to be of Asian descent) had the *HLA-B*15:02* allele. Similarly, a study of nevirapine-related rash and hepatitis in a Western Australian population showed an association between nevirapine hypersensitivity and the MHCII allele *HLA-DRB1*01:01* (138). In contrast, recent studies on Japanese and Sardinian populations have found associations of MHCI alleles in Sardinian (*HLA-Cw8/HLA-B14*) and Japanese (*HLA-Cw8*) HIV-infected subjects with nevirapine hypersensitivity. These observations suggest that a different immunogenetic basis for nevirapine hypersensitivity may exist in different populations (139, 140).

It is also important that both the positive and negative predictive values of the HLA association are formally estimated (as in the case of AHS) across large sample sizes and different genetic backgrounds. These data provide an indication of the cases of hypersensitivity that would be prevented compared with the number of individuals potentially inappropriately denied drug treatment when preclusion from drug treatment based on the possession of the hypersensitivity-associated HLA allele is instigated.

The utility of pharmacogenetic testing depends on several factors including specificity, sensitivity, reproducibility, precision, and ease of testing. As such, all of these factors need to be considered for estimating preprescription testing benefit and feasibility on a case-by-case basis. The generation of allotype-specific monoclonal antibodies for HLA testing has the potential to significantly accelerate HLA allotype screening when there is complete confidence in using the drug in the absence of the "offending" allotype (141).

Once the genetic associations have been confirmed, cost-benefit analysis should then be conducted to address issues such as (*a*) whether screening for an allele that is weakly associated with the hypersensitivity is worthwhile, (*b*) whether withholding of the drug from certain at-risk individuals imposes a lesser toll than that imposed by the hypersensitivity reactions prevented by the screening, (*c*) whether the cost of testing outweighs the cost of managing the hypersensitivity reactions, and (*d*) which populations would benefit most from the testing.

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