

Abacavir induces loading of novel self-peptides into HLA-B*57:01: an autoimmune model for HLA-associated drug hypersensitivity

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Background: Abacavir drug hypersensitivity in HIV-treated patients is associated with HLA-B*57:01 expression. To understand the immunochemistry of abacavir drug reactions, we investigated the effects of abacavir on 1) HLA-B*57:01 epitope-binding *in vitro* and 2) the quality and quantity of self-peptides presented by HLA-B*57:01 from abacavir-treated cells.

Design and methods: An HLA-B*57:01 specific epitope binding assay was developed to test for effects of abacavir, didanosine or flucloxacillin on self-peptide binding. To examine whether abacavir alters the peptide repertoire in HLA-B*57:01, a B-cell line secreting soluble HLA (sHLA) was cultured in the presence or absence of abacavir, peptides were eluted from purified HLA, and the peptide epitopes comparatively mapped by mass spectroscopy to identify drug-unique peptides.

Results: Abacavir, but not didanosine or flucloxacillin, enhanced binding of the FITC labeled self-peptide LF9 to HLA-B*57:01 in a dose dependent manner. Endogenous peptides isolated from abacavir-treated HLA-B*57:01 B-cells showed amino acid sequence differences compared with peptides from untreated cells. Novel drug-induced (DI)-peptides lacked typical carboxyl(C)-terminal amino-acids characteristic of the HLA-B*57:01 peptide motif and instead contained predominantly Isoleucine or Leucine residues. DI-peptides bind to soluble HLA-B*57:01 with high affinity that was not altered by abacavir addition.

Conclusion: Our results support a model of drug-induced autoimmunity in which abacavir alters the quantity and quality of self-peptide loading into HLA-B*57:01. Drug-induced loading of novel self-peptides into HLA, possibly by abacavir either altering the binding cleft or modifying the peptide loading complex, generates an array of neo-

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antigen peptides that drive polyclonal T-cell autoimmune responses and multi-organ systemic toxicity. © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

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Introduction

Therapeutic drugs are approved based on safety and efficacy in clinical trials. Although most drugs have favorable benefit-risk profiles, some treated individuals may still develop severe life threatening side effects. Understanding the biochemical and immunological mechanisms mediating drug-induced hypersensitivity and severe adverse drug reactions (ADR) is important for both treatment and prevention, as well as for identifying individuals at risk of developing adverse reactions before therapy. A number of immune-mediated or idiosyncratic drug reactions have been genetically linked with specific HLA Class I and Class II alleles. Drug hypersensitivity to abacavir [1–3] is linked to HLA-B*57:01. Severe cutaneous reactions (SCARs) associated with Class I HLA are seen with carbamazepine (CBZ) in some individuals expressing HLA-B*15:02 [4], B*15:11 [5], A*31:01 [6], and with allopurinol in HLA-B*58:01 [7]. Flucloxacillin causes Drug-Induced Liver Injury (DILI) in patients expressing HLA-B*57:01 [8].

Clinical syndromes of HLA-associated drug reactions vary widely depending on the severity of reactions and the target tissue/organ. Abacavir hypersensitivity involves multiple organ systems and within 1–2 weeks of initial treatment patients experience rash, fever, gastrointestinal symptoms, myalgias, fatigue, and in some cases respiratory symptoms. Overall, symptoms resemble graft-versus-host disease. Symptoms usually resolve with discontinuation of abacavir, however re-exposure or rechallenge results in rapid, severe, and life-threatening reactions.

Molecular mechanisms for HLA-associated drug reactions have not been clearly established. Several models [for review [9,10]] have been presented that include: 1) hapten/prohapten mechanisms where drugs or drug-metabolites can covalently or non-covalently bind to self proteins, peptides or HLA, thereby creating neo-antigens that are then processed and loaded into HLA molecules for presentation and activation of T-cells, 2) direct pharmacologic reversible interactions of drugs with immune receptors, called the p-i model [11], in which drugs such as CBZ [12] and sulfamethoxazole bind non-covalently to T cell receptors (TcR) or HLA-peptide complexes stimulating T-cells. Moreover, it is believed that drugs may also cause tissue damage and inflammation providing cofactors/costimulation that facilitate immune

cell activation [13]. Abacavir hypersensitivity may be driven by a hapten/prohapten-related mechanism as CD8+ T-cell responses require TAP/tapasin expression, antigen processing, and specific amino acids in the F pocket of HLA-B*57:01 for presentation to drug-specific T-cells to stimulate polyclonal responses [14]. *In-silico* models suggest that abacavir could interact with the F pocket of HLA-B*57:01 in the absence of peptide [15].

In this report, we tested abacavir by applying an *in vitro* B*57:01 peptide binding assay and examined the endogenous peptide repertoire presented by HLA-B*57:01 from abacavir-treated versus untreated cells using proteomic techniques. Here, we report that abacavir has dramatic effects on the quantity and quality of self-peptide loading to HLA-B*57:01 by possibly providing a pseudo-anchor position in HLA favoring neo-antigens in HLA or by modifying the peptide loading complex (PLC). Abacavir hypersensitivity therefore may result from this novel molecular mechanism, which drives autoimmune polyclonal T-cell responses.

Methods

Reagents

Abacavir sulfate (GSK and Santa Cruz Biochemicals), flucloxacillin (Apotex, UK), Didanosine (Bristol Myer Squibb). Synthetic peptides were made and purified to 98% purity by Peptide2.0 (Manassas, Virginia).

Peptide binding assay

To assess the ability of synthetically dened peptide epitopes to associate with HLA-B*5701, an assay based on inhibition of binding of the uorescent standard peptide LF9FITC (LSSPVT(K-FITC)KSF) was developed as previously published [16,17]. Binding events were determined using uorescence polarization. The assay was slightly modified for measuring drug interactions by replacing the addition of competitor peptides with the drug of interest.

Cell lines and transfectants

Soluble HLA-B*57:01 molecules are a trimeric complex consisting of a heavy chain comprised of $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains, a light chain ($\beta 2m$) and the peptide. The heavy chains of the sHLA class I molecules are truncated just

before the transmembrane domain and cytoplasmic domain [18]. The mammalian expression vector pcDNA3.1 carrying the sHLA-B*57:01 construct was used to express HLA-B*57:01 proteins in the class I negative EBV virus transformed lymphoblastoid cell line 721.221.

HLA peptide isolation

Soluble HLA-B*57:01 transfectants were cultured to high density in a hollow fiber bioreactor. After milligram quantities of sHLA was collected under non-treated conditions, Abacavir (10 µg/ml) was then added to the culture. Harvested proteins were purified using affinity chromatography. Peptides were released from the HLA complex by addition of 10% acetic acid under boiling conditions and separated from heavy chains and β₂m by passing them through a 3-kDa cut-off membrane filter (Millipore). Drug-treated and non-treated peptide pools were separated by reverse phase HPLC and fractions were collected. UV absorbance was monitored at 215 nm. Consecutive and identical peptide separations were performed for drug treated and untreated B*57:01 peptide-batches [see Supplement 1].

Liquid chromatography-mass spectrometry

Peptide fractions numbers 50, 60 and 70 obtained from reverse phase HPLC separations were analyzed by LC-MS as described in supplement 1.

Results

Abacavir enhances epitope binding to HLA-B*57:01

To evaluate effects of abacavir on HLA-peptide interactions, a specific peptide binding assay for HLA-B*57:01 was designed based on fluorescence polarization (FP) utilizing the FITC-labeled tracer peptide LF9FITC (LSSPVT(K-FITC)KSF). To test the feasibility of binding, competition of the unlabeled LF9 peptide against its labeled counterpart was shown (Fig. 1). The unlabeled LF9 peptide was titrated in 8 serial dilutions (10 nM–80 µM) and a logIC₅₀ value of 2.5 was determined, which is consistent with high affinity binding [16,17]. Abacavir was then tested in this assay over a 2 log range of concentrations and compared with didanosine, a related nucleoside analogue, and flucloxacillin, an antibiotic associated with liver injury in individuals bearing HLA-B*57:01 (Fig. 2a). Abacavir, in contrast to didanosine and flucloxacillin, increased LF9-FITC peptide binding in a dose-dependent manner, with effects starting at 10 µM and an overall half maximal effective concentration (EC₅₀) of 52 µM. In addition, the maximum plasma concentration (C_{max}) in patients from clinical trial is between 10–15 µM (Ziagen package insert), overlapping with concentrations that enhance epitope binding *in vitro*. These findings suggest that abacavir might have a direct

effect on the structure of HLA possibly by binding to the cleft to allow better access or more stable interactions of self-peptides with the HLA peptide binding site.

Abacavir treatment induces loading of unique self-peptides into HLA-B*57:01

The preceding results demonstrated that abacavir can increase self-epitope binding, suggesting that drug may alter quantitative and qualitative loading of self-peptides to HLA-B*57:01 in abacavir-treated cells. To characterize the effect of abacavir on the intracellular loading of endogenous peptide ligands, a 721.221 B-cell line producing sHLA-B*57:01 was cultured with and without abacavir and sHLA collected from the bioreactor outputs. Soluble HLA was affinity purified, peptides stripped and separated by preparative HPLC. Three of the matched HPLC fractions were then analyzed by LC-MS. The most abundant ions were compared between untreated and drug-treated samples and unique ions not present in controls were targeted for MS fragmentation. A number of sequence matches were found among unique and common peptides as listed in Table 1. Two examples of MS-MS fragmentation for drug unique ions are shown Fig. 3A, HSLPALIQI, and Fig. 3B, STIRLLTSL.

Overall, peptides common to both untreated and treated cells exhibited canonical P2 and C-terminal anchor motifs for B*57:01 including alanine (A), threonine (T) or serine (S) at the P2 position, and a C-terminal tryptophan (W), tyrosine (Y), or phenylalanine (F) [19,20]. In contrast, peptides unique to the drug-treated samples were biased at the C-terminus showing a high preference for Isoleucine (I) or Leucine (L) rather than W, Y, or F. This C-terminal impact of drug treatment did not extend to the P2 anchor; amino acids consistent with the B*57:01 motif (A, T or S) were observed. A diverse set of DI peptides from a variety of cellular proteins were identified including a sequence from a protein highly expressed in skin, the autoimmune target Bullous pemphigoid antigen-1, also called dystonin. As mentioned above, most unique peptides in drug treated samples had I or L at the C-terminus, but we identified a sequence containing a C-terminal W and A in P2. This peptide, TAGAHLRW, a desaturase found also in liver, was 8 amino acids in length. Interestingly, the same sequence with an additional I at the N-terminus, ITAGAHLRW, was present in both untreated and treated cells. Other peptides of interest from this set included the interferon induced GTP-binding protein MX-1.

Binding of novel drug-induced peptides to HLA-B*57:01

The appearance of unique peptides in cells treated with drug which were not present in untreated cells, suggested that these peptides may not bind to sHLA-B*57:01, and possibly require the presence of abacavir to be integrated into the complex. Several DI peptides were synthesized and then tested for binding to sHLA-B*57:01 in the

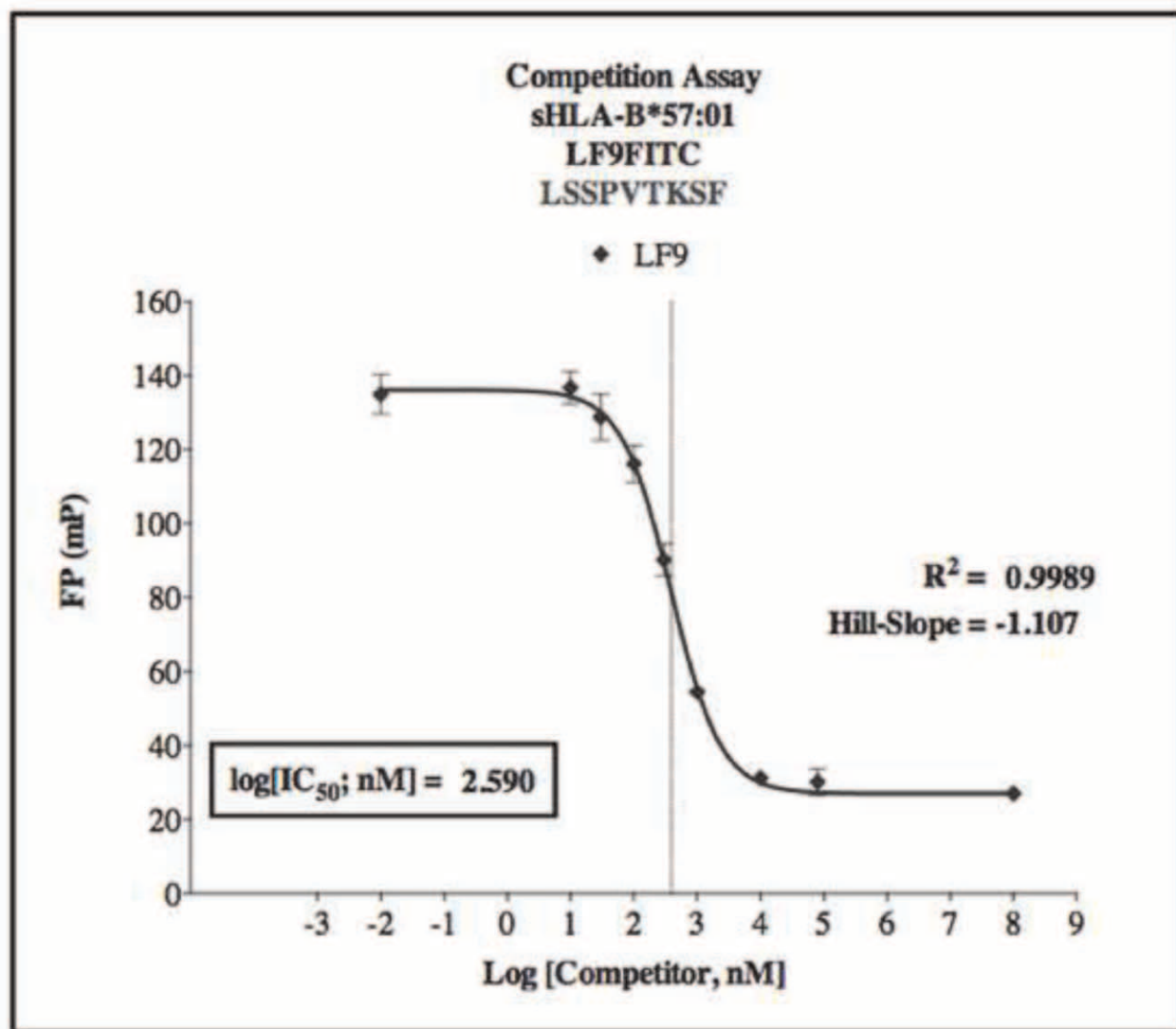


Fig. 1. (a) Fluorescence polarization based HLA-B*57:01 competition assay. Soluble HLA B*57:01 was derived from 721.221 B-cell transfectants and affinity purified before use in binding assays. The tracer is the self-peptide LF9 (LSSPVTKSF) from IgKappa substituted with a FITC-lysine residue at P6 that is not involved with direct HLA binding based on examination of the crystal structure (14). Serial dilutions of nonlabeled LF9 competitor were incubated with a constant concentration of heat activated sHLA-B*57:01, excess β m and the FITC-labeled tracer peptides until equilibrium was reached by monitoring changes in fluorescence polarization (FP) on an Analyst AD plate reader (Molecular Devices). The IC_{50} concentration of unlabeled competitor that produces pFITC binding half way between the upper and lower plateau is indicated.

binding assay. As reference, two DI-peptides, HSLPA-LIQI, and STIRLLTSL, that contain I/L at the C-terminus could strongly compete in the HLA-B*57:01 peptide binding assay (Fig. 4A,B), with $\log IC_{50}$ values consistent with high affinity binding. Addition of abacavir did not substantially shift the IC_{50} of the DI-peptides thus showing no dramatic change in the affinity of the peptide for sHLA-B*57:01, indicating that once folded, sHLA might provide a higher affinity for these peptides than in the intracellular PLC.

Discussion

We report here that abacavir enhances peptide binding in a sHLA-B*57:01 specific assays and induces loading of novel self-peptides into sHLA-B*57:01 expressing cells treated with drug. Novel DI-peptides carried distinct changes in the HLA-binding motif characteristic of sHLA-B*57:01 epitopes, consisting of I or L at the C-terminus, instead of the consensus F-pocket binding amino-acids (W, F or Y). DI-peptides bind with high affinity to monomeric sHLA-B*57:01 in the absence of

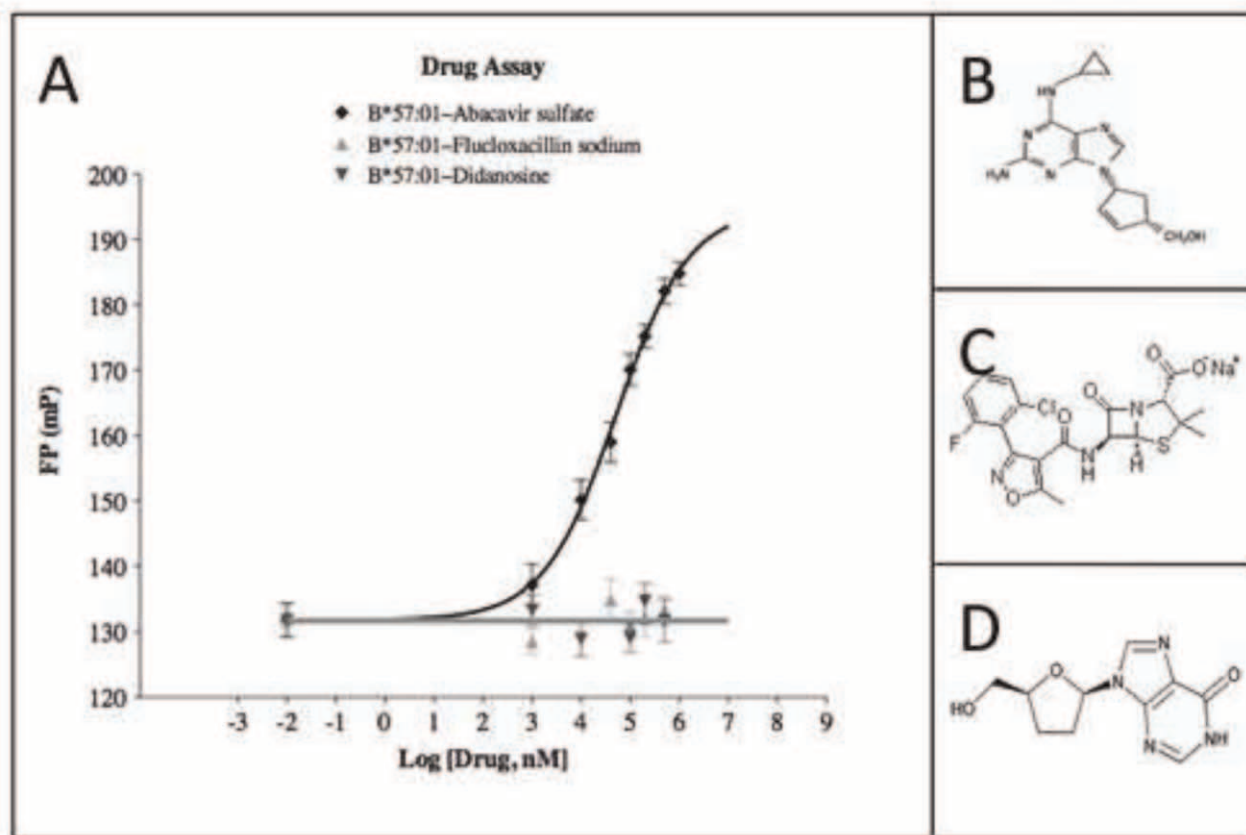


Fig. 2. (a) Effect of abacavir sulfate, didanosine and flucloxacillin on LF9FITC binding to sHLA-B*57:01. Structures for drugs are shown in (a) abacavir, (c) flucloxacillin, and (d) didanosine. To monitor drug effects on peptide binding, abacavir, didanosine or flucloxacillin were added over a wide concentration range and FP measured at equilibrium. The increase in fluorescence polarization represents an increase in epitope binding to HLA.

drug, and abacavir does not change their IC_{50} . These results support a model of drug-induced autoimmunity where drugs can alter the quality and quantity of the endogenous peptide repertoire in HLA to generate neoantigens and drive polyclonal T-cell responses to self-epitopes.

Abacavir induced autoimmunity helps to explain the multi-organ systemic hypersensitivity reactions in which DI-peptides would be expressed on HLA in many tissues, including skin and the GI system, to drive T cell expansion and serve as targets for CD8+ effector cells. Once drug is discontinued DI-peptides would disappear, reactive T cell pools would contract and differentiate to T memory cells. Re-exposure to abacavir would again generate DI-peptides in APCs and tissues leading to rapid expansion of T memory cells and effectors to cause severe and some cases life threatening reactions.

Our data indicate that drug hypersensitivity to abacavir may involve two processes. Abacavir, but not didanosine and flucloxacillin, enhanced binding of the self-peptide ligand (LF9) carrying a consensus motif (C-terminal-phenylalanine, F) for HLA-B*57:01, in a dose-dependent

manner which did not require drug metabolism. The half-maximal effective concentration of abacavir was $52 \mu\text{M}$, which seems to suggest a low affinity binding interaction. Although flucloxacillin is associated with liver toxicity in HLA-B*57:01 individuals, it may require metabolism to form a reactive intermediate to bind protein or HLA. Metabolites of abacavir created by alcohol dehydrogenase [21] may have a similar activity to enhance peptide binding and have potential to create covalent protein adducts. Direct effects of abacavir on binding suggest that the drug could alter the structure of HLA to affect peptide binding in the HLA cleft, including the possibility that abacavir remains stably bound to HLA and peptide in the complex. If abacavir remains in the complex, peptides with HLA-B*57:01 consensus motifs could adopt conformations that are different in the presence of drug compared with peptide in HLA alone and be recognized as neoantigens by T cells. Functionally, it is feasible that increased peptide binding to HLA-B*57:01 of epitopes with consensus-matching motifs could break tolerance if other co-stimulatory or inflammatory cofactors are present. Alternatively, as supported by our peptide analysis, the presence of abacavir during peptide loading may substantially change

Table 1. HLA-B*57:01 Common and Abacavir-Induced Unique Peptides in HPLC fractions 50, 60, 70.

Sequence	Fraction	Score	Measured m/z (z)	MH ⁺ Matched	Error (ppm)	Accession#	Protein Name
Abacavir-Induced Unique Peptides (C-terminus- I/L)							
GTITGVDVSm ^a	50	10.82	498.2409 (2)	979.476	3.1	P62314	Small nuclear ribonucleoprotein SM
KSVDPENNP TL	50	7.49	607.3080 (2)	1213.606	2.3	Q9Y510	Transportin-3
TAGAHLRW	60	19.39	456.2471 (2)	911.485	2.5	O00767	Acyl-CoaA desaturase
ASSSQITHI	60	13.75	478.2650 (2)	955.521	2.1	Q6PL18	AAA domain containing protein
NTVELRVKI	60	13.18	357.8901 (3)	1071.652	3.4	P04844	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2
STSDIITRI	60	12.73	503.2833 (2)	1005.558	1.8	P49585	Choline-phosphate cytidyltransferase A
IVKn*KPVEL	60	11.90	520.8219 (2)	1039.651	1.4	Q8IZJ1	Netrin receptor UNC5B
RTFETIVKQQI	60	9.79	454.9306 (3)	1362.774	2.4	P20591	Interferon-induced GTP-binding protein Mx1
GTINIVHPKL	60	9.79	364.5581 (3)	1091.657	2.4	Q9Y617	Phosphoserine aminotransferase
LSTDVAKTL	60	8.35	474.2748 (2)	947.541	1.6	Q03001	Bullous pemphigoid antigen 1
GTAPVNLNI	60	8.14	449.7539 (2)	898.499	1.4	Q6UN15	Pre-mRNA 3'-end-processing factor FIP1
KTIEDDLVSAL	70	10.16	602.3281 (2)	1203.647	1.8	Q9Y606	tRNA pseudouridine synthase A, mitochondrial
ASITPGTILI	70	10.03	493.3010 (2)	985.593	1.9	Q02878	60S ribosomal protein L6
STIRLLTSL	70	9.54	502.3111 (2)	1003.615	0.3	P493768	T-complex protein 1 subunit gamma
LHLLVIR	70	9.09	432.2954 (2)	863.582	1.1	Q9Y4F4	Protein FAM179B
HSLPALIQI	70	6.37	496.3015 (2)	991.593	2.2	Q8N8D1	Programmed cell death protein 7
Common Peptides (C-terminus - F, W, Y)							
IAVKNVNSY	50	19.21	344.1951 (3)	1030.568	2.7	Q8N2W9	SUMO-protein ligase PIAS4
YVPEDEDLK	50	17.88	554.2660 (2)	1107.520	3.9	Q8WUD4	Coiled-coil domain containing protein 12
VTAEIDRQY	50	15.43	547.7791 (2)	1094.548	3.0	Q99873	Protein arginin N-methyltransferase 1
VTKTVSND SF	50	14.42	549.2785 (2)	1097.547	2.2	P55209	Nucleosome assembly protein 1-like 1
ISQPASGNTF	50	13.24	511.2522 (2)	1021.495	2.2	Q14157	Ubiquitin-associated protein2-like
ITAGAHLRW	60	21.68	342.1937 (3)	1024.569	-2.1	O00767	Acyl-CoA desaturase
VAKVGQYTF	60	18.65	506.7774 (2)	1012.546	1.3	Q86Y39	NADH dehydrogenase 1 alpha subcomplex subunit11
VTYKNV PNW	60	18.31	560.7940 (2)	1120.579	1.9	P62826	GTP-binding nuclear protein Ran
TVYYPKEEV	60	17.03	574.7867 (2)	1148.562	3.4	P19387	DNA-directed RNA polymerase II subunit RPB3
KTVTAMD VVY	60	11.15	563.7952 (2)	1126.581	1.6	P62805	Histone H4
HAIPLRSSW	60	10.66	533.7942 (2)	1066.579	1.8	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit b-1
KTVTAmDVVY	60	10.00	571.7925 (2)	1126.581	1.6	P62805	Histone H4
LSAFLPARF	70	13.39	511.2965 (2)	1021.583	2.7	Q9NUQ2	1-acyl-sn-glycerol-3-phosphate acyltransferase 8

^am: Oxidized Methionine; n: Deamidation.

HLA-B*57:01 peptide motif: P2-(A,T,S), P9-Carboxy-terminus-(F,W,Y).

the structure of the peptide receptive HLA-B*57:01 groove in the PLC, fostering distinct peptide requirements for high affinity binding. Structural studies are required to address conformations of peptides and abacavir in HLA-B*57:01.

The second molecular process, probably related to the first, is the ability of drug to promote loading of a more distinct repertoire of self-peptides in HLA-B*57:01 in cells. These neo-peptides range in length from 9 to 11 amino acids and lack the C-terminal amino acids W, F or Y, characteristic of HLA-B*57:01 motifs that interact with the F pocket [19]. Instead, most of the novel peptides contain aliphatic I/L at the C-terminus, and have the preferred, canonical amino acids in the P2 position [19]. We believe the small set of peptides identified here are representative of a larger number of drug unique peptides carrying unusual C-terminal amino acids, along with other self-peptides with correct HLA-B*57:01 motifs that are enhanced by abacavir. In addition, if abacavir is metabolized to form protein adducts then haptenized peptides could also be presented in HLA. However, we have not, as yet, identified covalent drug-peptide adducts in the peptide fractions. Of note, HLA-B*57:01 epitopes with C-terminal I/L or V have been reported from a variety of microorganisms [20,22], although not as endogenous peptides from human proteins. Another

study examining HLA bound peptides from CBZ-treated cells expressing HLA-B*15:02 did not find differences in the peptide repertoire from drug treated cells compared to control cells [23].

Unexpectedly, DI-peptides bind to sHLA with high affinity and addition of abacavir did not modify their IC₅₀ values. Therefore, DI-peptides are capable of binding HLA-B*57:01, but either have a low affinity during *in vivo* loading or have limited access to the PLC in cells. Abacavir may provide a non-covalent hydrophobic anchor substitute during loading in the PLC by bridging between the C-terminal aliphatic I/L and the F-pocket amino acids such as serine-116 or aspartic acid-114, thereby stabilizing the neo-epitope. Abacavir may also modify tapasin or TAP interactions in the PLC [24] to allow DI-peptide binding where normally these peptides are excluded. TAP/tapasin and antigen processing are required for presentation of abacavir to CD8+ T-cells and drug presentation maps to HLA amino-acid serine-116 (14). By this mechanism, abacavir would not be required to remain in a complex with HLA and peptide after loading, and therefore might contribute transiently to peptide binding *in vivo*. Binding of DI-peptides to recombinant sHLA-B*57:01 *in vitro* without drug would be compatible with this model, suggesting that the folded conformation of the sHLA in the binding assay provides a

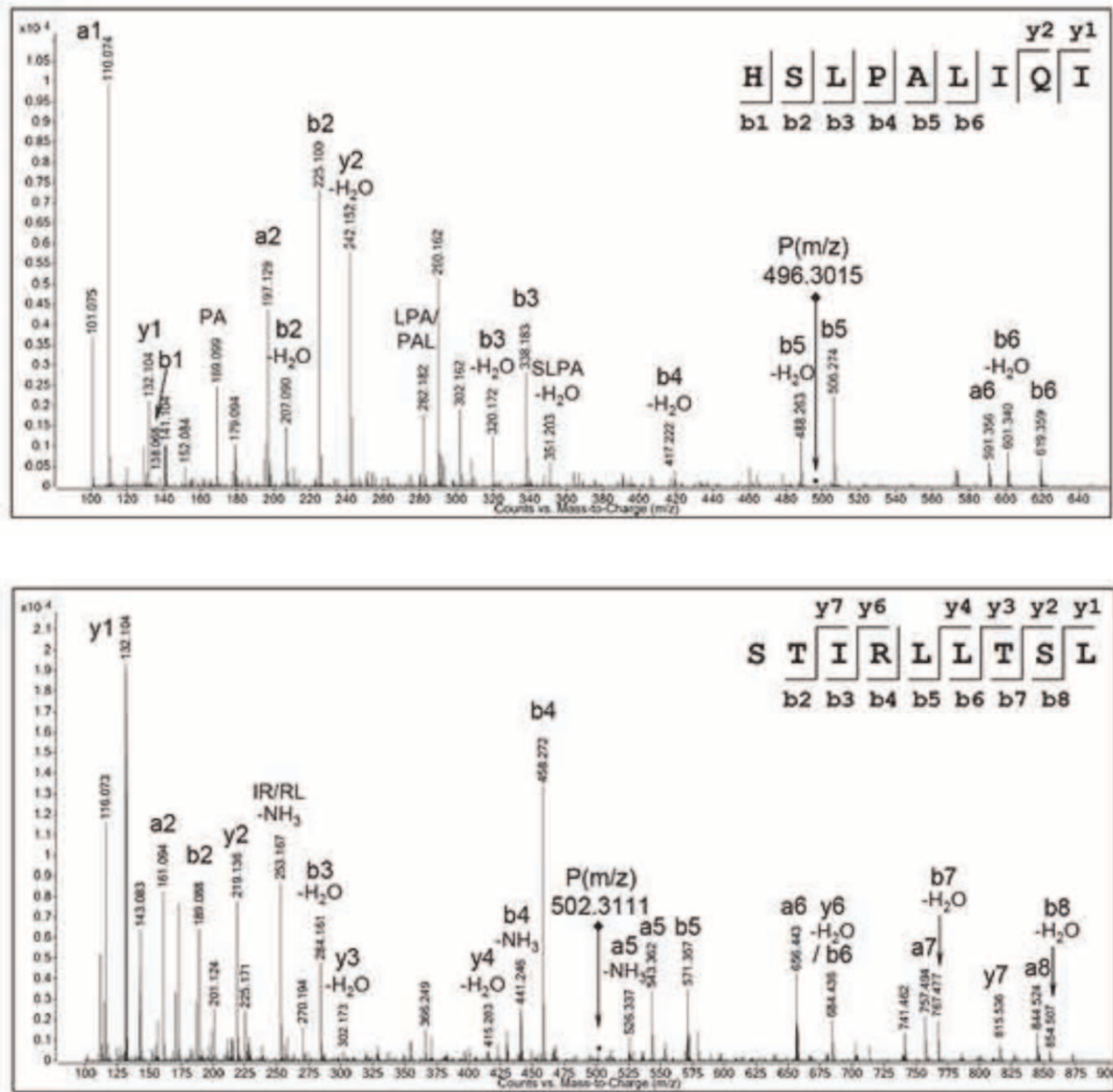


Fig. 3. Examples of MS-MS fragmentation spectra from abacavir-unique peptides from fraction 70. (a) Peptide MW 990.5868 HSLPALIQI, (b) Abacavir-unique peptide MW 1002.6061 STIRLLTSL.

higher affinity pocket than the peptide receptive conformation of the cleft in the PLC. It is possible that with some peptides abacavir may remain in the HLA complex. Alternatively, abacavir could also impact peptide repertoires by modifying other steps in peptide processing, delivery, and loading. Further biochemical and structural studies are needed to define abacavir interactions with HLA.

Our data support a model of drug-induced autoimmunity as a consequence of abacavir exposure. Peptides with I/L in the C-terminus may not bind strongly to HLA-B*57:01 *in vivo* and therefore be absent during thymus

development. Thus, T-cell clones recognizing peptide epitopes with these characteristics would not go through clonal deletion. Upon drug exposure DI-peptides and some peptides with consensus motifs will be loaded into HLA. This generates neoantigens and stimulates naive T cells to undergo a primary immune response, and possibly activates some cross-reactive memory T cells. Initial responses to DI-peptides may occur in lymphoid tissues either by direct presentation by resident antigen-presenting cells or by Langerhans cells migrating from skin or from other tissues. Novel self-peptide presentation along with co-stimulation would promote activation and expansion and then migration of effector T-cells to tissues

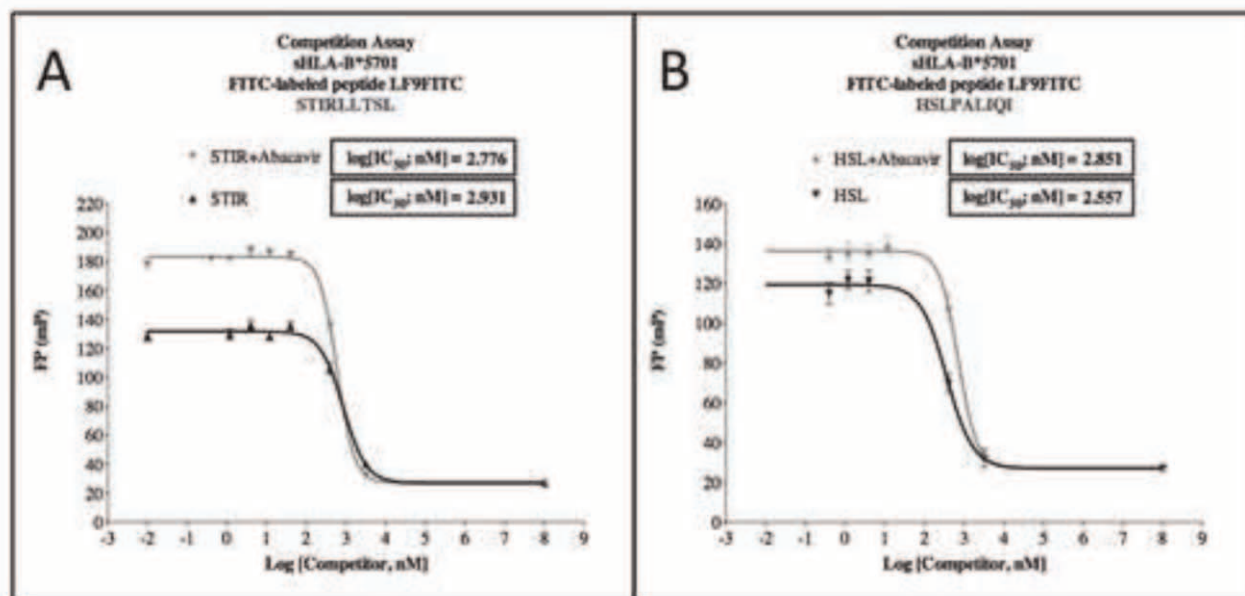


Fig. 4. Competitive binding evaluation of HLA-B*57:01 DI peptides (a) HSLPALIQI and (b) STIRLLTSL with and without abacavir. IC₅₀ values for two DI peptides were determined applying serial dilutions of nonlabeled HSL and STIR competitor, respectively, in the presence and absence of 200 μ M of abacavir. Results showed the expected increase in reference LF9FITC peptide binding signal with abacavir. No significant difference in inhibition between experiments in both cases could be observed.

where antigens and drugs are present in high amounts, such as skin. Interestingly, we identified a peptide from Bullous pemphigoid antigen 1/dystonin that is present in skin and could be a potential target for autoimmunity. Presumably many other self-antigens could be targeted by similar mechanisms. For drugs that induce liver injury with HLA associations, T-cells similarly could be generated centrally in lymphoid tissue and migrate to liver to attack cells with drug- or metabolite-dependent liver-specific epitopes. Discontinuation of drug therapy would remove the source of self-peptides in HLA in APCs and in tissues and curtail T-cell auto-reactivity.

Furthermore, we identified a peptide corresponding to the interferon inducible protein, MX1. HIV infection is associated with high levels of interferon. In these circumstances, and in the presence of abacavir, cells responding to interferon may become targets for autoimmune T-cell recognition. Further experiments are required to verify that DI-peptides are recognized by T cells from abacavir hypersensitive patients.

Many HLA-B*57:01 individuals are able to tolerate abacavir and not show hypersensitivity (1), thus it is clear that HLA-B*57:01 is required but not sufficient for drug hypersensitivity, supporting a role for additional host cofactors or inflammatory stimuli. In the absence of co-stimulation, T-cells may become or remain tolerant to DI-peptides. Cofactors could include direct drug innate effects or tissue damage causing secondary inflammation, or infection such as by HIV itself in the case of abacavir.

Alternatively, tolerant patients may have been previously exposed to DI-peptides centrally in the thymus leading to T cell deletion or peripherally to anergize T cells. Another mechanism could involve suppressor activities of T regulatory cells on the auto-reactive T cells.

The effect of abacavir on HLA loading of novel self-peptides with potential to drive autoimmunity may represent a model of drug hypersensitivity for other drugs with genetic associations with HLA. Analysis of drug interactions with other alleles and drug combinations examining HLA peptide binding along with molecular approaches to identify unique epitopes in HLA induced by drug exposure will help to unravel molecular mechanisms and targets for drug reactions. These methods may also help to predict novel HLA linkages for new drugs to prevent potential unforeseen toxicities. Furthermore, we suggest that altered loading of novel self-peptides with modifications in HLA-anchor motifs through perturbations in the PLC may occur not only as a consequence of drug exposure, but also as a process that contributes to some autoimmune disorders.

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preliminary MS analysis. L.L. expressed and purified sHLA proteins and performed HLA binding assays. M.G., A.D.R. performed and R.B. developed, supervised and performed the HLA peptide/competition binding assays.

M.A.N., R.B., M.T.B. and W.H.H. conceived, planned, supervised and analyzed experimental data. D.H.M. and J.W. were involved in the conception, planning and review of data. M.A.N. wrote the first draft of the paper and M.T. B., D.H.M., W.H.H. and R.B. reviewed and edited the manuscript to the final version.

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Conflicts of interest

There are no conflicts of interest.

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