

HLA-B*1502-bound peptides: Implications for the pathogenesis of carbamazepine-induced Stevens-Johnson syndrome

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Background: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) can involve MHC-restricted presentation of a drug or its metabolites for T-cell activation. HLA-B*1502 tightly associated with carbamazepine (CBZ) induced these conditions in a Han Chinese population. **Objective:** We sought to identify HLA-B*1502-bound peptides that might be involved in CBZ-induced SJS/TEN. **Methods:** Soluble HLA-B*1502 was used to identify bound peptides in the presence and absence of CBZ by using liquid chromatography–tandem mass spectrometry. Peptide-binding assays were performed to detect the specific interaction between the HLA molecule and the identified peptides. Mass spectra were compared to detect CBZ-modified peptides. **Results:** We identified more than 145 peptides bound to HLA-B*1502. In 13 of 15 peptides examined, we functionally confirmed their specificity with binding assays. Preferable uses of these peptides at the anchoring residues P2 and P9 were similar to those observed in other HLA-B alleles in the Han Chinese population. However, the preferable use of serine residues at the nonanchoring position (P) 5, P6, P7, and P8 appeared to be unique for the B*1502 peptides. No specific CBZ-modified peptides were detected when we compared the mass spectra of peptides detected in the presence or absence of the drug. **Conclusion:** Noncovalent interaction between a drug and an HLA complex might contribute to cytotoxic T cell–mediated cell death in patients with SJS/TEN. **Clinical implications:** An understanding of pharmacologic interaction of drugs with an HLA complex might lead to safer

drugs that avoid SJS/TEN. (J Allergy Clin Immunol 2007;119:1016–1022.)

Key words: Adverse drug reactions, carbamazepine, drug antigen, epitope peptide, HLA-B, Stevens-Johnson syndrome

Stevens-Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), are the most severe forms of cutaneous adverse drug reactions (cADRs).¹ They are characterized by a rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement and skin detachment.² More than 100 drugs can cause SJS/TEN. The most common culprits in white subjects are nonsteroidal anti-inflammatory drugs, anticonvulsants, allopurinol, and sulfonamides and other antibiotics.³ In Taiwan carbamazepine (CBZ), a commonly prescribed anticonvulsant, is the leading culprit and accounts for 25% of all cases of drug-induced SJS/TEN.⁴

The mechanisms by which drugs cause cADRs are not well understood. Immune reactions can be involved in SJS/TEN because a rechallenge with the same agent typically results in a severe symptom within 2 to 3 days. In addition, cytotoxic T cells infiltrate the skin lesions of patients with SJS/TEN,⁵ and the composition of T cells in the blister fluid of patients shows a predominance of the CD8⁺ phenotype.⁶ A hallmark of SJS/TEN is widespread apoptosis of keratinocytes⁷ triggered by cytotoxic T cells. These observations indicate that CD8⁺ T cell–mediated cytotoxic responses are the major events in SJS/TEN.⁸

Evidence also supports the view that severe cADRs involve MHC-dependent presentation of a drug or its metabolites for T-cell activation.⁶ Schnyder et al⁹ reported the direct MHC-dependent presentation of sulfamethoxazole to the T cells. Naisbitt et al¹⁰ reported that MHC class II might present CBZ and is recognized by the T-cell receptors on CD4⁺ T cells in patients with CBZ-induced hypersensitivity syndrome.

Because drugs and their metabolites are small chemicals and therefore unlikely to trigger an immune reaction by themselves, a hapten hypothesis has been proposed.¹¹ According to this hypothesis, specific drugs or metabolites act as haptens. They covalently bind to proteins or peptides and are presented by MHC molecules after cellular

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Abbreviations used

cADR: Cutaneous adverse drug reaction
 CBZ: Carbamazepine
 sHLA-B*1502: C1R-soluble HLA-B*1502
 SJS: Stevens-Johnson syndrome
 TEN: Toxic epidermal necrolysis
 tHLA-B*1502: C1R-transmembrane HLA-B*1502

processing, resulting in the HLA-restricted T-cell activation. Another hypothesis, the p-i concept (direct pharmacologic interaction of drugs with immune receptors), proposes that drugs can directly and noncovalently bind to matching T-cell receptors with MHC molecules.¹²⁻¹⁴ Both hypotheses suggest that drug-induced cADRs involve the interaction of specific MHC molecules, T-cell receptors, and drug-modified antigens. However, the exact MHC molecules involved were unknown until recently, when HLA-B*5701 was reported to be associated with hypersensitivity caused by abacavir¹⁵ and our results showed that HLA-B*1502 was the MHC molecule strongly associated with CBZ-induced SJS/TEN.⁴ We and others subsequently demonstrated that the specific MHC molecules associated with genetic susceptibility to different severe cADRs are drug, phenotype, and/or ethnicity specific.¹⁶⁻²⁰ The nearly 100% association of HLA-B*1502 with CBZ-induced SJS/TEN implies that HLA-B*1502 is not only a genetic marker but also a participant in the pathogenesis of the disease. Further support for this role is derived from our results from gene fine mapping, with which we narrowed the susceptible region to within 86 kb of the area where HLA-B is the only known gene.¹⁷

The purpose of our study was to identify HLA-B*1502-bound peptides and to investigate whether the hapten hypothesis or p-i concept is involved in the pathogenesis of HLA-B*1502-restricted CBZ-induced SJS.

METHODS

The human B-lymphoblastoid cell line Hmy2.C1R (CRL-1993; American Type Culture Collection, Manassas, Va) is HLA class I deficient, having intact antigen processing and presentation pathways. Murine w6/32 mAb (HB95, American Type Culture Collection) is an IgG2a specific for a monomorphic HLA-A, HLA-B, and HLA-C determinants.

Construction and generation of C1R-soluble and transmembrane HLA-B*1502 stable clones

C1R-soluble HLA-B*1502 (sHLA-B*1502) stable clones were established, as previously described by Prilliman et al.²¹ HLA truncation was achieved by means of PCR amplification of full-length HLA-B*1502 cDNA with the forward primer (5'-GGGCAAGCTTGGACTCAGAATCTCCCCAGACGCCGAG-3') (boldface and italics denotes the *Hind* III cutting site) and the reverse primer (5'-CCGCGAATTCATCTCAGGGTGAG-3')²² (boldface and italics denotes the *Eco* RI cutting site; underlining denotes the stop codon) and cloned into a pcDNA3.1(+) vector (Invitrogen, Carlsbad, Calif).

By using an electroporation system (BioRad Laboratory, Hercules, Calif), the plasmid DNA was transfected to C1R cells and selected with 600 µg/mL G418 (Sigma, St Louis, Mo). Soluble HLA secretion was tested with sandwich ELISA in which the w6/32 mAb served as the capture antibody. Anti-B₂-microglobulin-horseradish peroxidase (Dako, Carpinteria, Calif) served as the detecting antibody, and soluble HLA-B*1501 complex (Pure Protein LLC, Oklahoma City, Okla) was the standard.

C1R-transmembrane HLA-B*1502 (tHLA-B*1502) stable clones were established by means of PCR amplification with the forward primer (5'-GGACTCAGAATCTCCCCAGACGCCGAG-3') and the reverse primer (5'-CTGGGGAGGAAACACAGGTCAGCATGGGAAC-3')²³ and cloned into a pcDNA3.1(+) vector. We selected tHLA stable clones by detecting the protein expression using anti-HLA class I antibody (number 555552; BD Biosciences, Bedford, Mass) with flow cytometry.

Production and purification of soluble HLA-B*1502 molecules

The procedures for HLA production and purification were similar to those described by Yague et al.²⁴ C1R-sHLA-B*1502 stable clones were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mmol/L L-glutamine, 100 µg/mL streptomycin, and 100 U/mL penicillin. CBZ (Novartis, Basel, Switzerland) was added to the culture at a final concentration of 50 µg/mL for 48 hours. The media with and without CBZ were subjected to affinity chromatography with w6/32 antibody-coupled cyanogen bromide-activated Sepharose 4B (Amersham, Piscataway, NJ). Soluble HLA molecules were eluted with 0.2 N acetic acid, heated at 70°C for 10 minutes, and subjected to centrifugal filtration with a 3-kD cutoff (Amicon Ultra-4; Millipore).

Analysis of peptides with liquid chromatography-tandem mass spectrometry

Our general schema for peptide identification was the one described by Hickman et al.²⁵ In addition, half of a sample was labeled with formaldehyde, which globally labeled the N-terminus and the ε-amino group of lysine residue²⁶ and added 28 d on the labeling site, resulting in a shift of product-ion spectra. Peptides identified both with and without formaldehyde labeling were regarded as candidate peptides. The peptide mixture was separated by using reverse-phase capillary liquid chromatography (Magic C18AQ, 75 µm × 11 cm; Microm BioResources, Inc, Auburn, Calif) at a flow rate of 200 nL/min. The eluent was directly analyzed with ion-trap mass spectrometry (Finnigan LCQ Deca XP; Thermo Scientific, San Jose, Calif). A survey scan followed by 3 collision-induced dissociation events was used. The results were analyzed with a software program (Sequest in Bioworks 3.1, Thermo Scientific). A database was downloaded from the Web site of the National Center for Biotechnology Information (for *Homo sapiens*). Mass spectra in the presence and absence of CBZ were compared by using a software program (Qual browser module of Xcalibur 1.3; Thermo Scientific).

HLA class I peptide-binding assay

We tested the abilities of the synthetic peptides to reconstitute HLA complexes using the methods outlined by Nilges et al.²⁷ Five of these peptides were chosen because they were reported in a previous publication,²⁸ and 10 were chosen for their high abundance, uniqueness, or both. Endogenous peptides were stripped from HLA-B*1502 complexes of C1R-tHLA-B*1502 cells with citric acid treatment (pH 2.9). After neutralization, the cells were incubated with β₂-microglobulin (4 µg/mL; Chemicon International, Temecula, Calif), protein transport inhibitor (GolgiStop; 1 µL/mL,

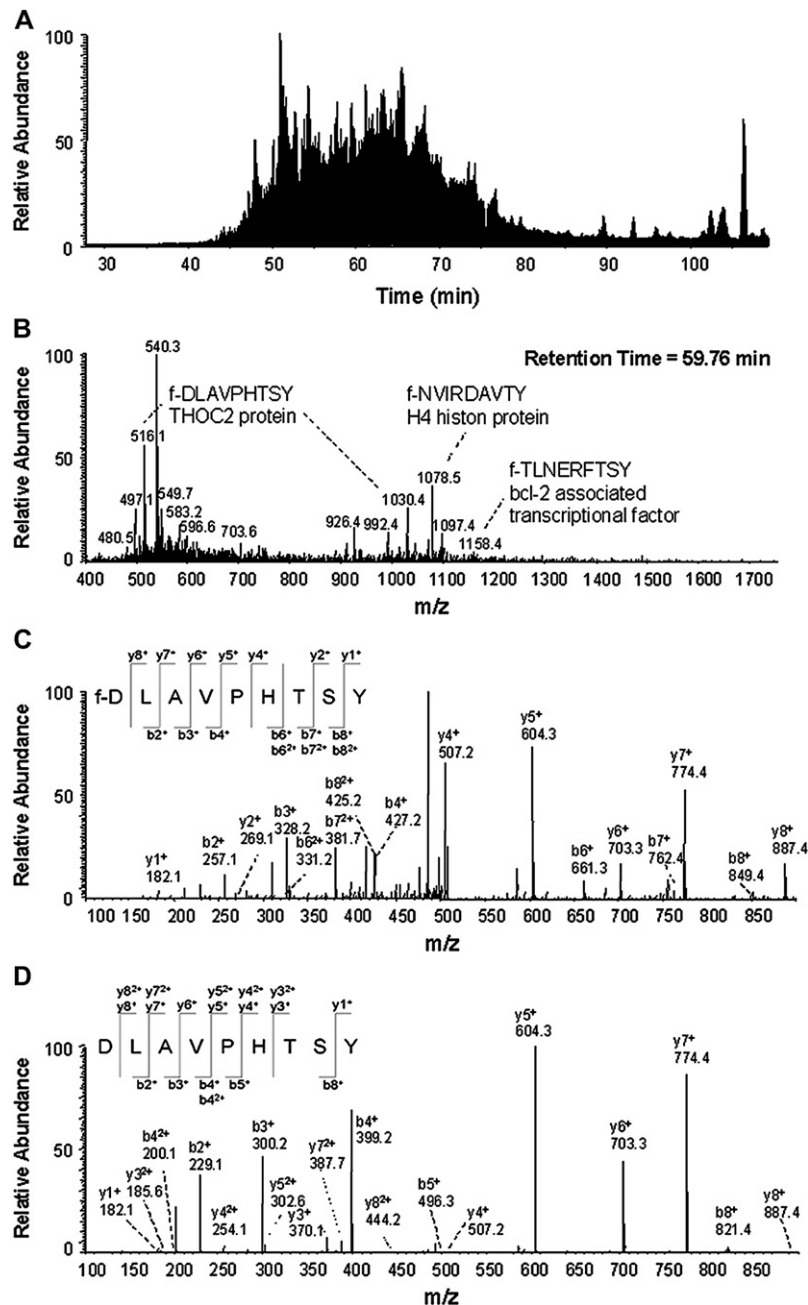


FIG 1. HLA-B*1502 peptides identified with liquid chromatography–tandem mass spectrometry. **A**, Chromatogram illustrates change in the total ion current. **B**, Ions at mass-to-charge ratio (m/z) 516.1 and 1030.4 were predicted to have the same sequence with different charges. **C**, Product-ion spectrum of the ion at m/z 516.1. The ion was identified as the peptide labeled with formaldehyde, f-DLAVPHTSY. **D**, Product-ion spectrum of the same peptide without labeling, DLAVPHTSY.

BD Biosciences PharMingen, San Diego, Calif), and individual synthetic peptides with serial concentration of 10^{-4} to 10^{-7} mol/L (95% purity, Angene and Mission Biotech, Taiwan) for 3 hours and detected by using anti-HLA class I antibody (no. 555552; BD Biosciences). They were then analyzed with flow cytometry. Controls were non-acid-treated C1R-tB*1502 cells and acid-treated cells without peptide addition. Reconstitution ability was expressed as the ratio of the linear fluorescence channels of acid-treated cells with and without peptide.

RESULTS

Identification of soluble HLA-B*1502-bound peptides with mass spectrometry

Fig 1, A, shows the total ion current chromatogram of HLA peptides, and Fig 1, B, shows the mass spectrum recorded at the retention time of 59.76 minutes. The ion at a mass-to-charge ratio of 516.1 was identified as the

TABLE I. The amino acid preference at each residue position of the HLA-B*1502 peptides

Amino acid	Frequency of occurrence								
	1	2	3	4	5	6	7	8	9
A	2	32	65	24	38	14	32	67	3
C	1	0	0	0	0	0	1	0	0
D	37	0	9	46	33	10	3	2	0
E	53	3	3	35	6	8	16	33	4
F	50	5	37	8	13	3	4	11	70
G	1	1	9	64	44	76	14	6	0
H	9	0	9	2	14	26	37	7	0
I	8	26	59	10	21	22	14	5	0
K	0	0	23	29	4	12	5	10	0
L	3	148	34	15	11	12	12	4	5
M	9	9	7	2	0	1	3	0	10
N	46	2	5	3	20	10	11	1	2
P	0	16	1	76	65	15	15	2	0
Q	10	51	5	11	27	27	11	31	0
R	0	0	23	16	1	8	4	3	0
S	83	5	13	12	44	65	81	67	10
T	48	9	10	4	18	60	82	69	9
V	2	86	15	35	31	25	48	43	3
W	0	0	11	0	1	0	0	1	0
Y	32	1	56	2	3	0	1	31	234

Boldface numbers indicate the top 3 preferred amino acids at each residue position.

TABLE II. Amino acid residue preference of peptides identified in the HLA-B alleles

Allotype	Peptide position									References
	1	2	3	4	5	6	7	8	9	
sB*1502	S E F	L V Q	A I Y	P G D	P G S	G S T	T S V	T S A	Y F	Present study
tB*1502		L V Q P	*Y R F	P E D G	F H A	D I	V L M	Y	Y F M	28
B*0705	A R	P	*A L M	G E R					L F	29
B*0801		L P	K R	E V Q	K R		E		L	30
B*1501	I	Q L		P E G D	G L F T	V T G I	V T L I M	Y V T	Y F	21, 31, 32
B*1503		Q K	*F Y R	P G E D		R V			Y F	33
B*1512		Q L M	*K F R	P E D G	T			Y	Y F	34
B*2704		R	Y F K L N	K Q P	K A	Y V I	L I T		Y L F	35
B*2706		R	Y K F L V				V K T		L	36
B*3501	M	* P A V	*I L F	K D E G P	*D I V	*I Q K	*V N E	E Q V T	Y F M L I	37, 38
B*3901		R H	*A D I	D E G P K	*V Y I	I V L	N Y F	S K R	L	39
B*4001		E	*A V I	*P K D	*L I V	*K N P	I V L Y M	K R Q	L	31
B*4006	G R	E P	*F I L V	*E G P	*V I L	I N	*Y V L	K S	V A P	31
B*4403	A S	E							Y F	40
B*4601		M I	*K R N	D E V	P I G	S A	E G	V A	Y F	32
B*4801		Q K M	F L I Y V	G R D	I V				L	41
B*5101	I L V Y D	A P G W F	*I L M	*G V I	*V T G	N I L K Q	K Q R E	T	I V	31
B*5102		P A G	Y F V L I	G E K L	*V Q N	I N Q T	R E Q K	T R Y	I V	31
B*5401	M F	P	*F M R	G	V				A	42
B*5502	A	P	*R M Y	H	I		V	M S	A	42
B*5601	A	P	Y	G T E	V I		V	A S M	A L	42
B*5801	K R I	A S T G	*G T I	*P E K	*V I L	I V L F	L Y M N	N R K T	F W Y	28, 31
B*6701	A	P	*F M Y	G K E	V	M			L	42

Boldface letters indicate the major anchoring residues.

sB*1502, Secreted form of HLA-B*1502, present study; tB*1502, transmembrane form of HLA-B*1502.²⁸

*No residue preferences (over 5 amino acids at the same position).

peptide labeled with formaldehyde, f-DLAVPHTSY (Fig 1, C).

The product-ion spectrum of intact peptide DLAVPHTSY is shown in Fig 1, D. Compared with Fig 1, D, the b-ions in

Fig 1, C, were shifted 28 d for a single charge ion, and the y-ions were not shifted. Peptides confirmed in this way greatly reduced the possibility of false-positive results.

Peptides identified

In the absence of CBZ, 145 peptides were considered correct because they were identified both with and without formaldehyde labeling. The identified peptides and the possible sources are listed in Table E1 in the Online Repository at www.jacionline.org. The identified peptides were mainly 8 (9%), 9 (79%), and 10 (10%) amino acids long, calculated on the basis of 145 peptides. These lengths corresponded to the general lengths of MHC class I peptides. According to detection frequencies, the most abundant peptides typically contained 6, 7, or 8 proline residues. However, these proline-rich peptides failed to bind the HLA-B*1502 molecule (see below), and therefore they were excluded from calculation of preferable amino acid residues.

Sharing of specific anchoring residues

We calculated the frequency of specific amino acid occurrence at each position of the 145 peptides by multiplying the detected numbers of each peptide. Among the 394 detected numbers, preferences were strongest for L (35.9%) at anchoring residue P2, followed by V (22.1%) and Q (12.7%) at the same position. The amino acid residues at the C-terminal (P9 or P10) of these peptides were predominantly aromatic Y (64.5%) and F (19.5%, Table I).

Because HLA-B*1502 is tightly associated with CBZ-induced SJS/TEN, whereas other HLA-B alleles in Han Chinese subjects are not, we compared the amino acid preferences of peptides isolated from 22 available HLA-B alleles in Han Chinese patients to determine the specificity of HLA-B*1502 peptides (Table II).^{21,28-42} The preferred residue (L, V, or Q at the P2 anchoring position) was also found in other HLA-B alleles. The preferred residue (Y or F at the P9 position) was found in 9 HLA-B alleles, and 4 of them belong to HLA-B15. Of note, almost all of the HLA-B alleles had the similar amino acid preference (ie, D, E, G, or P at the P4 residue).

Preferable use of serine at nonanchoring residues of B*1502 peptides

Because anchoring residues at P2 and P9 of B*1502 peptides were also found in other HLA-B alleles, the specific interaction between B*1502 and CBZ was unlikely to have occurred at the conserved anchoring positions. Table I shows the frequencies of the amino acid residues of B*1502 peptides, and Table II summarizes the preferable amino acid uses in peptides of other HLA-B alleles.

The preferred residues at P5, P6, P7, and P8 were serine, and those at P6, P7 and P8 were threonine. The frequencies of serine residues at P5, P6, P7, and P8 were 11%, 17%, 19%, and 17%, respectively, and the frequencies for threonine residues were 14%, 19%, and 16%. Preferred threonine use was not specific for B*1502 because it was also found in the B*1501 allele, which is not associated with CBZ-induced SJS/TEN. However, the preferred use of serine at positions 6, 7, and 8 was unique for B*1502

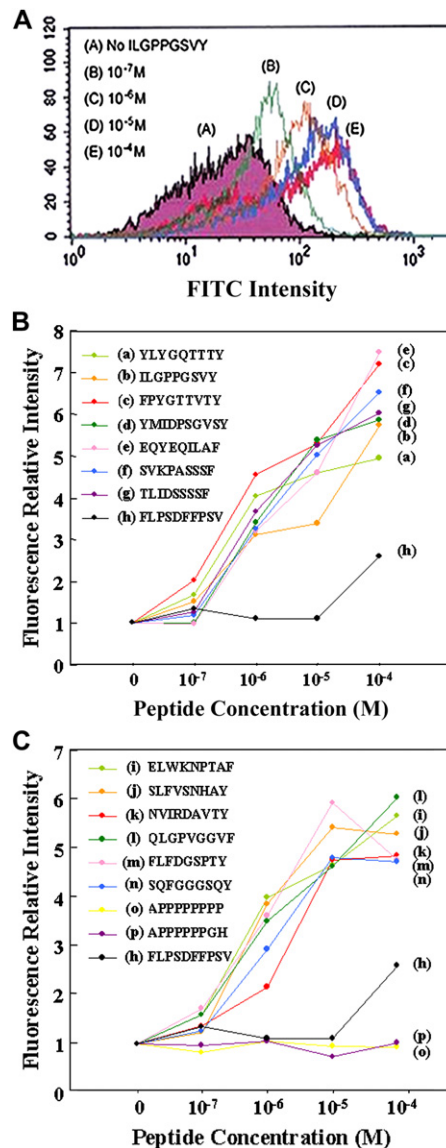


FIG 2. HLA-B*1502 peptide-binding assay. **A**, ILGPPGVS was the positive control. **B**, Assays for 5 peptides we and others²⁸ identified (a-e) and 2 serine-rich peptides (f and g). **C**, Assays for 6 peptides (i-n) and 2 proline-rich peptides (o and p) we identified. In Fig 2, B and C, a peptide derived from the HLA-A2-restricted HBV epitope was the negative control (h).

because none of the peptides associated with other HLA-B alleles had the same pattern (Table II). The identified peptide SVKPASSSF (supplementary, detection frequency 8) carried 3 consecutive serines. Peptides NLHSSSTF (detection frequency 6) and TLIDSSSF (detection frequency 2) carried 4 consecutive serine residues that were not found in other HLA-B peptides.

High affinity of identified peptides with HLA-B*1502

Reconstitution of the HLA complex depended on the affinity/concentration of the peptides, and the 5 peptides that we and previous investigators identified had high

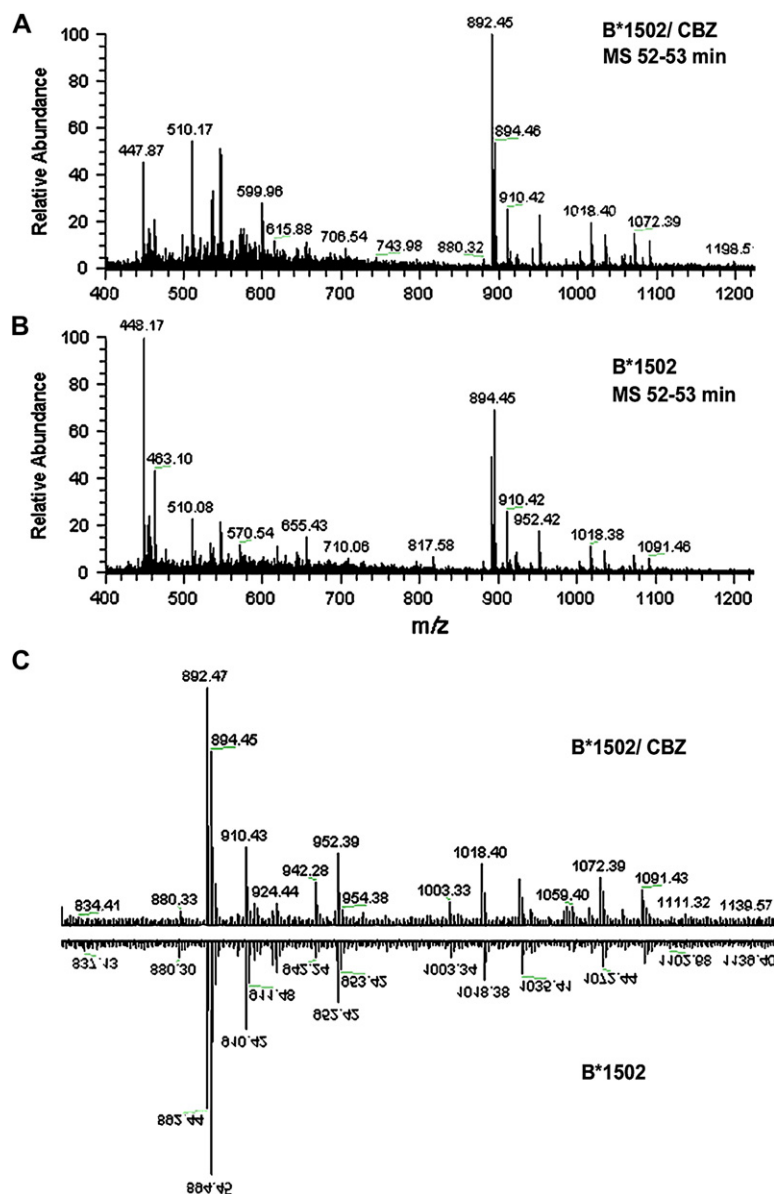


FIG 3. Mass spectra of soluble HLA-B*1502-bound peptides with (A) and without (B) CBZ. Peptide compositions were compared at 1-minute intervals. Signals between 52 and 53 minutes were compared. To compare the graphs in detail, each graph was divided into 3 or 4 parts, and each part was zoomed out to align the signals (C).

affinities for HLA-B*1502 (Fig 2, B).²⁸ Another 8 peptides were identified only by using our method; these included 2 polyserine peptides that contained 3 to 4 consecutive serine residues, which also had strong affinities for HLA-B*1502 (Fig 2, B). However, the polypyrroline peptides (APPPPPPP and APPPPPPGH) that we tested in the peptide-binding assay did not show affinity for HLA-B*1502 (Fig 2, C).

Similar HLA-B*1502-bound peptides identified in the presence of CBZ

The peptides identified in the presence and absence of CBZ have the same pattern. The preferable uses of amino

acids were similar with or without CBZ. Serine-rich peptides identified in the CBZ-free samples were also found in the CBZ-treated samples.

No detection of CBZ covalently modified peptides

The general patterns of peptides eluted from liquid chromatography were similar, except for a peak that appeared at 83 minutes in the CBZ-treated sample but not in the CBZ-free sample (data not shown). This peak was proved to be CBZ when we compared the product-ion spectrum from tandem mass spectrometry with that of pure CBZ. The mass spectra were further examined in

detail (Fig 3). Fig 3, C, is an example of a comprehensive analysis of 60 mass spectra. The image shows complete matching of the 2 spectra with and without CBZ. In summary, no new peaks and no ions with mass shift were detected. This result suggested that no covalently drug-modified peptides were formed in the presence of CBZ.

DISCUSSION

The preferable use of serine residues at the nonanchoring positions P5, P6, P7, and P8 appeared to be a unique feature of the HLA-B*1502-bound peptides. Furthermore, several peptides carried 3 and even 4 consecutive serine residues at these positions. In searching the SYFPEITHI database,⁴³ which provides updated information about identified MHC epitopes, we found no peptides with 4 consecutive serine residues. SHIPQSSSY, which contains 3 consecutive serine residues, was found in HLA-B*1518, an allele present in 0.5% to 1.8% of Han Chinese.⁴⁴ Whether persons who carry the HLA-B*1518 allele are at increased risk for CBZ-induced SJS/TEN is currently unknown. The role of serine residues in these HLA-B*1502-bound peptides is not clear at this time.

Although 13 of 15 peptides examined were functionally confirmed in the binding assay, 2 proline-rich peptides that we tested failed to show specific binding to HLA-B*1502. These peptides could be detected with and without formaldehyde labeling. However, their abundance might have been artificially high because they were eluted in a broad retention time instead of in a sharp peak. We do not know why the nonspecific, proline-rich peptides were detected in our system.

Barber et al²⁸ previously reported 7 HLA-B*1502-bound peptides when they used transmembrane HLA-B*1502 as the bait and when they determined amino acid sequences by means of Edman degradation. The low number of peptides they identified was most likely because they fractionated the peptide mixture with HPLC, and only well-separated peptides were subjected to Edman degradation. Despite differences in our methods, we observed 5 of the 7 peptides they identified, and the preferences for amino acid residues at positions P2, P4, and P9 were comparable in both studies. The 2 peptides that were not detected in our system might be explained by the low binding affinity with soluble HLA-B*1502 compared with that with the transmembrane HLA-B*1502 used in their study. Alternatively, these 2 peptides might not exist in the C1R cell line we used but are present in the 721.221 cell line used by Barber et al.²⁸

In our comparison with and without CBZ, we found no CBZ-modified peptides. Liquid chromatography–tandem mass spectrometry might not have been sensitive enough to detect all peptides present in low abundance. The antigenic peptides might have been specific to only keratinocytes, the targets cells in SJS/TEN. Moreover, the *in vitro* use of soluble HLA-B*1502–producing B cells might not have reflected the *in vivo* conditions in which CBZ is metabolized to more than 30 metabolites

in the liver. One of the reactive metabolites, CBZ 10,11-epoxide has the potential to covalently bind to protein. Although we did not test the CBZ 10,11-epoxide directly, incubating CBZ with B cells should produce epoxide because the key enzyme, microsomal epoxide hydrolase, is known to be present in the lymphocytes.^{45,46} Despite the experimental limitations, our results implied that the interaction between peptides and CBZ or its metabolites might have been due to noncovalent binding and that the drug dissociated from peptides under the experimental condition of the acid treatment. Taken together, our results seem to favor the p-i concept that the noncovalent interaction of drug, peptides, and HLA-B molecules of antigen-presenting cells and T-cell receptors triggers drug hypersensitivity.

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[Q16] **TABLE E1.** The sHLA-B*1502-bound peptides identified

Peptide sequence	Source protein	Detection frequency	Gene ID
APPPPPPP	HLA-B-associated transcript 3	32	7917
SQFGGSQY	Eukaryotic translation initiation factor 3	12	8664
ELWKNPTAF	Ubiquitin-conjugating enzyme E2C isoform	11	11065
*YLYGQTTTY	DNA topoisomerase II, α isozyme	10	7153
LPPPPPPP	Zinc finger protein 318	10	24149
IPPPPPPP	WIRE protein	10	147179
SLFVSNHAY	Aldolase A	9	226
SLDAPHSTY	Syntaxin binding protein 2	9	6813
DALVITHY	Zinc finger protein 259	9	8882
TAAAVQVF	Solute carrier family 26	9	65010
NVIRDAVTY	H4 histone family 2	8	8370
SVKPASSSF	LAS1-like	8	81887
*FPYGTTVTY	Complement component receptor 2	7	1380
SLVGITSAY	Protein phosphatase 1, regulatory subunit	7	4659
HPPPPPPP	Human R3H domain protein 1	7	23518
SPPPPPPP	KIAA1429, isoform CRA_b	7	25962
EVAPAGASY	Glioma tumor-suppressor candidate region gene	7	29997
FLSFPTTKTY	A ₂ -Globin	6	3040
SLREDQQSF	Linker for activation of T cells family	6	7462
NLHSSSTF	Ubiquitin associated protein 2-like	6	9898
ELAKQITSY	Chaperonin containing TCP1, subunit 8	6	10694
TVIDSQTHY	P24B protein transport domain	6	23423
SQYQGVVVY	Nuclear import 7 homolog	6	51388
VPPPPPPP	SFRS protein kinase 2 isoform b	6	57466
FLFDGSPTY	Fatty-acid synthase	5	2194
*YMIDPSGVSY	Proteasome subunit, α type	5	5684
EVILHHEY	Transmembrane and coiled-coil domain family	5	23023
MLYGTITEF	Mob1, MPS1 binder kinase activator-like 1B	5	55233
NQIRPGLQY	Spermatid perinuclear RNA-binding protein	5	55342
TVIPNSHVY	Brix domain containing 5	5	80135
FLAPLVTEY	Hypothetical protein FLJ30655	5	132320
KPPPPPPP	Mediator of RNA polymerase II transcription	5	219541
FIKDGSTY	ICB-1 γ	5	
DQMISRIEY	Casein kinase 1, α 1	4	1452
IVADHVASY	MHC, class II, DQ	4	3117
NIAGDGSVF	Karyopherin α 2	4	3838
NLLDPGSSY	PRKC apoptosis regulator	4	5074
EAIIPGSVY	Regulator of nonsense transcripts 1	4	5976
DSYKVTHY	Pre-B-cell colony enhancing factor	4	10135
FLKPDDIQY	Splicing factor 3B, subunit 1	4	23451
NQKPPSAEY	Microtubule-actin crosslinking factor 1	4	23499
ELAPSLPSY	COMM domain containing protein	4	51122
DTYKAITY	Transmembrane protein 70	4	54968
FIGEFTTSY	Copine family member	4	151835
YMFVDENTF	CXX1 protein	4	
ILTVGGTAY	Cytochrome c oxidase subunit VIIa polypeptide	3	1347
FVAEQKVEY	Eukaryotic translation initiation factor 4 γ	3	1981
TAYPGQTQY	Eyes absent homolog 3	3	2140
QLGPVGGVF	Fatty-acid synthase	3	2194
FFESFGDL	Hemoglobin β	3	3043
SVRGGDEVY	Human NF- κ B P52 bound to Dna	3	4791
SISKALVAY	Ribosomal protein S16	3	6217
YLFEPHSGEEY	SFRS protein kinase 1	3	6732
YLLPDTKAY	Tumor rejection antigen (gp96) 1	3	7184
NQIESKAAY	Ubiquitin-specific peptidase	3	8237
EVLPIHAY	FEM-1 homolog B	3	10116
NVYDFKTTY	SSU72 RNA polymerase II CTD phosphatase homolog	3	29101
TQALASVAY	ABI gene family, member 3	3	51225
DLAVPHTSY	THOC2 protein	3	57187
DLLGVGAEY	KIAA0892 protein	3	

TABLE E1. (Continued)

Peptide sequence	Source protein	Detection frequency	Gene ID
NTIDPSHPM	Alanyl-tRNA synthetase	2	16
TVYMGKVTF	Dynactin 1	2	1639
*EQYEQILAF	Eukaryotic translation initiation factor 2	2	1968
QVAPVANSY	Erythroblastosis retrovirus oncogene homologue	2	2114
TLIDSSSSF	Guanine-nucleotide exchange factor	2	2889
EVIPYTPAM	Heme oxygenase	2	3162
SVINPGAIY	Integrin α 4	2	3676
DLLLVRQY	mutS homolog 2	2	4436
SVIGASSQF	Phosducin-like protein	2	5082
TLRSEGFDTY	Proliferating cell nuclear antigen	2	5111
MIIGGGMAF	Phosphoglycerate kinase	2	5230
TTAPSHAKF	Protein phosphatase 1, regulatory subunit	2	5514
QLTGQTPVF	Ribosomal protein L11	2	6135
SVMTLVSTY	Signal sequence receptor	2	6747
NAAFPFLAY	Transferrin receptor	2	7037
HVVAASKAM	Eukaryotic translation initiation factor 3	2	8663
YVHDDGRVSY	Glia maturation factor, γ	2	9535
YPVEIHEY	Nuclear DNA-binding protein	2	10438
FAFEHSEY	NULP1	2	22980
QERPSVTSY	Anaphase promoting complex subunit 7	2	51434
SQQPASGVAY	Zinc finger RNA binding protein	2	51663
DVSPVVSTY	Hypothetical protein FLJ12649	2	79649
TVIPNSHVYY	Brix domain containing 5	2	80135
ALAPAGFSY	Dot1-like, histone H3 methyltransferase	2	84444
SIREAGGAF	ATPase inhibitory factor	2	93974
DAYELKHAL	Annexin A5	1	308
YPQPPSQSY	Annexin VII isoform 2	1	310
DLLDIRSEY	Annexin A11	1	311
DLYPHVVGf	Rho GTPase activating protein 1	1	392
FLYGNEVSY	Complement component receptor 2	1	1380
TLRDIETFY	Eukaryotic translation initiation factor 4A	1	1973
TLRDIETFY	Eukaryotic translation initiation factor 4A	1	1973
EIREAANAM	Acyl-CoA synthetase long-chain family member 4	1	2182
LVTVKPCYNY	Glypican 4	1	2239
YPTQPGQGY	Fusion (involved in t[12;16] in malignant liposarcoma)	1	2521
EQVPQQPTY	Growth factor receptor-bound protein 2	1	2885
HLLEKSNIY	Lymphoid-specific helicase	1	3070
TLLPSAEESY	Major Histocompatibility complex, class II, DQ	1	3117
DLFGRDLSY	Heterogeneous nuclear ribonucleoprotein F	1	3185
TLRDPGSQL	Insulin-like growth factor 2 receptor	1	3482
HVINTGNSM	Integrin	1	3676
FVKGWGAEY	SMAD, mothers against DPP homolog 2	1	4087
FPALPGSSY	CCR4-NOT transcription complex, subunit 2	1	4848
VLIDVGTTY	Prefoldin subunit 5	1	5204
EVYHKSMTL	Proteasome α 5 subunit	1	5686
NVADLHEKY	Proteasome subunit, β type	1	5693
EIMREGSAF	RAD21 homolog	1	5885
YLGEFSITY	Ribosomal protein S15	1	6209
FPYDYSASEY	Stearoyl-CoA desaturase	1	6319
TFNEPGSEY	SWI/SNF related, matrix associated protein	1	6597
NLFGGEPLSY	Transferrin receptor	1	7037
HPTVVISAY	Chaperonin containing TCP1, subunit 3	1	7203
*ILGPPGSVY	Ubiquitin-conjugating enzyme E2E	1	7325
TILGPPGSVY	Ubiquitin-conjugating enzyme E2E	1	7325
MPYLEHESF	CDC7 cell division cycle	1	8317
DLLREEY	Carboxylesterase 2 isoform 1	1	8824
QQNPQPLIF	Aryl hydrocarbon receptor interacting protein	1	9049
SVHQPGSHY	Postsynaptic protein CRIPT	1	9419
SQLVAGVAF	Chaperonin containing TCP1, subunit 7	1	10574
ELAQTTRTAY	Chaperonin containing TCP1	1	10694

TABLE E1. (Continued)

Peptide sequence	Source protein	Detection frequency	Gene ID
LSLDQSTWN	KIAA0922, isoform CRA_a	1	23240
SLAYLVNTY	Mitochondrial carrier homolog 2	1	23788
TLQKQSVVY	Pre-mRNA processing factor 31 homolog	1	26121
TLTPIIQEY	Programmed cell death 4	1	27250
SILEKTSAY	Chromosome 17 open reading frame 27	1	29102
HQAIHTIEY	ATPase, H ⁺ transporting, lysosomal V0 subunit	1	50617
TVKGVSLSSY	Hypothetical protein LOC51012	1	51012
CTPLFMNAY	APAF1 interacting protein	1	51074
SLSAPLTSY	Leucyl-tRNA synthetase	1	51520
EERADLIAY	Cytochrome c, somatic	1	54205
VGYRVATF	Dolichyl-phosphate mannosyltransferase	1	54344
SQRYSGAY	Coiled-coil-helix-coiled-coil-helix domain containing 3	1	54927
TAYVGSAMV	Pleckstrin homolog domain containing protein	1	55041
TLQDSRTNTAY	Pleckstrin homology domain containing, family B member	1	55041
TLNVSTTRY	Ankyrin repeat domain	1	55608
SQSQPSSQY	FERM domain containing 4A	1	55691
FIPTGHSL	Septin	1	55752
TILHSTGQY	Nucleoporin	1	57122
DFLAALEY	PI 3,4,5-trisphosphate-dependent Rac exchanger	1	57580
HQITVLHVV	Elongation of long chain fatty acids	1	60481
EQYESFVKF	Chromosome 1 open reading frame 108	1	79647
NQVIFPVS	Mak3 homolog	1	80218
SVVAHLSTY	Actin like protein	1	81569
HLRETQAQY	Protein phosphatase 1, regulatory subunit	1	84687
EQAPPVIYY	ALKB, alkylation repair homolog 6	1	84964
EVNPNTRVM	ORM1-like 3	1	94103
HPIQTQAQY	La ribonucleoprotein domain family	1	113251
GYTLAAI	Lactamase	1	114294
FVNPGVVEF	Tetratricopeptide repeat domain 14	1	151613
MVVGTGTSL	Keratinocyte-associated protein 2	1	200185
SLRSEDNAVY	Immunoglobulin heavy chain	1	
SLRTDDNAVY	Immunoglobulin heavy chain	1	

*These peptides were also identified from a previous publication: Barber LD, Percival L, Arnett KL, Gumperz JE, Chen L, Parham P. Polymorphism in the alpha 1 helix of the HLA-B heavy chain can have an overriding influence on peptide-binding specificity. *J Immunol* 1997;158:1660-9.