

## Direct interaction between HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson syndrome

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**Background:** Increasing studies have revealed that HLA alleles are the major genetic determinants of drug hypersensitivity; however, the underlying molecular mechanism remains unclear. **Objective:** We adopted the HLA-B\*1502 genetic predisposition to carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) as a model to study the pathologic role of HLA in delayed-type drug hypersensitivity. **Methods:** We *in vitro* expanded CBZ-specific cytotoxic T lymphocytes (CTLs) from patients with CBZ-induced SJS/TEN and analyzed the interaction between HLA-B and CBZ analogs based on CTL response, surface plasmon resonance, peptide-binding assay, site-directed mutagenesis, and computer modeling. **Results:** The endogenous peptide-loaded HLA-B\*1502 molecule presented CBZ to CTLs without the involvement of intracellular drug metabolism or antigen processing. The HLA-B\*1502/peptide/ $\beta_2$ -microglobulin protein complex showed binding affinity toward chemicals sharing 5-carboxamide on the tricyclic ring, as with CBZ. However, modifications of the ring structure of CBZ altered HLA-B\*1502 binding and CTL response. In addition to HLA-B\*1502, other HLA-B75 family members could also present CBZ to activate CTLs, whereas members of the HLA-B62 and HLA-B72 families could not. Three residues (Asn63, Ile95, and Leu156) in the peptide-binding groove of HLA-B\*1502 were involved in CBZ presentation and CTL activation. In particular, Asn63 shared by members of the B75 family was the key residue. Computer simulations revealed a preferred molecular conformation of the

5-carboxamide group of CBZ and the side chain of Arg62 on the B pocket of HLA-B\*1502.

**Conclusions:** This study demonstrates a direct interaction of HLA with drugs, provides a detailed molecular mechanism of HLA-associated drug hypersensitivity, and has clinical correlations for CBZ-related drug-induced SJS/TEN. (J Allergy Clin Immunol 2012;■■■■:■■■■-■■■■.)

**Key words:** Carbamazepine, drug hypersensitivity, HLA, Stevens-Johnson syndrome, toxic epidermal necrolysis

T cell-mediated delayed-type drug hypersensitivity appears as diverse clinical manifestations, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). SJS and TEN are life-threatening drug hypersensitivities that are characterized by serious blistering reactions in the skin and mucous membranes with systemic complications and multiple organ involvement.<sup>1</sup> SJS/TEN lesions are induced by the migration of circulating skin-homing cytotoxic T lymphocytes (CTLs) that are activated, proliferate, and release cytotoxic proteins to induce keratinocytes apoptosis.<sup>2,3</sup>

Recently, increasing HLA alleles are reported to associate with different kinds of drug hypersensitivity. For example, HLA-B\*1502 is strongly associated with carbamazepine (CBZ)-induced SJS/TEN,<sup>4</sup> HLA-B\*5801 with allopurinol-induced SJS/TEN/hypersensitivity syndrome (HSS),<sup>5</sup> HLA-B\*5701 with abacavir hypersensitivity<sup>6</sup> or flucloxacillin-induced liver injury,<sup>7</sup> and HLA-DRB1\*1501 with lumiracoxib-induced hepatotoxicity.<sup>8</sup> Nevertheless, in spite of the strong genetic associations, how a specific HLA allele plays its pathologic role in the initiation of the immune reaction of drug hypersensitivity is not yet well characterized.

Several hypotheses have been proposed to explain the interaction of HLA, drugs, peptides, and T-cell receptors (TCRs). The hapten/prohapten hypotheses propose that a chemically active drug/metabolite forms a covalent bond with an endogenous peptide and then is intracellularly processed and presented by the particular HLA as seen in the classical peptide antigen pathway.<sup>9</sup> For example, HLA-B\*5701 was reported to present abacavir to T cells through a tapasin- and processing-dependent pathway.<sup>10</sup> By comparison, the p-i concept (direct pharmacologic interaction of a drug with immune receptors) implies that the direct and reversible interaction of drugs with TCRs or peptide-loaded HLA initiates immune reactions.<sup>11</sup> However, there are very few examples proving the hypotheses.

The genetic predisposition of the HLA-B\*1502 allele to CBZ-induced SJS/TEN in Han Chinese patients is the strongest HLA-disease association reported thus far ( $P = 1.6 \times 10^{-41}$ ; odds ratio, 1357; 95% CI, 193.4-8838.3).<sup>4,12</sup> The same association has also

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**Abbreviations used**

AED:	Antiepileptic drug
APC:	Antigen-presenting cell
B-LCL:	EBV-transformed B-cell line
CBZ:	Carbamazepine
CTL:	Cytotoxic T lymphocyte
ECBZ:	Carbamazepine 10,11-epoxide
ESL:	Eslicarbazepine acetate
5HB:	5H-dibenzazepine
HSS:	Hypersensitivity syndrome
LIC:	Licarbazepine
LTT:	Lymphocyte transformation test
MCSS:	Multiple copy simultaneous search
OXC:	Oxcarbazepine
SJS:	Stevens-Johnson syndrome
SPR:	Surface plasmon resonance
TCL:	T-cell line
TCR:	T-cell receptor
TEN:	Toxic epidermal necrolysis

been replicated in other populations, particularly in patients from Southeast Asian countries, where the HLA-B\*1502 allele is frequent.<sup>13-15</sup> Recently, we reported that a prospective genetic screening of HLA-B\*1502 before CBZ treatment could reduce the incidence of CBZ-induced SJS/TEN.<sup>16</sup> It is interesting to note that CBZ-induced SJS/TEN has also been reported to associate with the different members of the HLA-B\*75 family in populations in regions where HLA-B\*1502 is rare.<sup>15,17-19</sup> The HLA association is phenotype specific because CBZ-induced maculopapular eruption/HSS has been reported to associate with HLA-A\*3101 in different populations.<sup>12,20,21</sup> Because HLA alleles are the major genetic determinants of drug hypersensitivity, here we adopted the model of the association between HLA-B\*1502 and CBZ-induced SJS/TEN to study the pharmacological mechanism of HLA in patients with drug hypersensitivity.

**METHODS****Clinical samples**

We enrolled 20 patients with CBZ-induced SJS/TEN (19 patients with CBZ-induced SJS and 1 patient with CBZ-overlapping SJS and TEN, all of whom were HLA-B\*1502 positive) and 4 CBZ-tolerant control subjects who were HLA-B\*1502 positive and had been administered CBZ for at least 3 months without any cutaneous adverse reaction from Chang Gung Memorial Hospital and National Taiwan University Hospital, Taiwan (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). All patients with SJS/TEN were accessed through review of photographs, pathologic slides, and medical records by 2 dermatologists in this study. Approval was obtained from the institutional review board, and informed consent was obtained from each participant.

**Chemicals, cell lines, and CBZ-specific T cells**

We purchased CBZ, carbamazepine 10,11-epoxide (ECBZ; the reactive metabolite of CBZ), oxcarbazepine (OXC; a second-generation drug to CBZ), eslicarbazepine acetate (ESL; a third generation drug to CBZ), licarbazepine (LIC; the active metabolite of OXC or ESL), 5H-dibenzazepine (5HB; also known as iminostilbene), and gabapentin (a nonaromatic antiepileptic drug [AED] used as the control drug) from Sigma-Aldrich (St Louis, Mo). These chemicals are illustrated in Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). We used EBV-transformed B-cell lines (B-LCLs), an HLA class I-deficient lymphoblastoid cell line (C1R), and a keratinocyte cell line

(KERTr) as antigen-presenting cells (APCs). We overexpressed different HLA-B proteins in C1R or KERTr cells by transfecting full-length or soluble cDNAs using Effectene (Qiagen, Hilden, Germany) or electroporation. CBZ-specific T-cell lines (TCLs) were obtained by culturing the patients' PBMCs with CBZ (25 µg/mL) for 10 to 14 days, and the expanded T cells were then restimulated with irradiated (50 Gy) autologous B-LCLs and CBZ for approximately 4 to 5 cycles. The T-cell clones were obtained by means of serial dilution.<sup>22</sup> CTL TCLs were sorted by using FACSAria (BD, Franklin Lakes, NJ).

**T-cell proliferation and cytotoxic assays**

We used the TCLs for pulsing experiments, cross-reactivity, HLA restriction, and antigen processing. Briefly, the TCLs ( $10^4$  cells) were incubated with irradiated APCs ( $10^3$  cells) and drugs for 48 hours, and the proliferation of TCLs was measured based on incorporation of tritiated thymidine (Perkin-Elmer, Waltham, Mass) for 16 hours. For the fixation assay, the autologous B-LCLs were pretreated with 0.25% paraformaldehyde for 30 minutes. Proliferative responses of T cells are shown as stimulation indices (in counts per minute in the drug-treated group/counts per minute in the vehicle-treated group). For the cytotoxicity assay, we first labeled APCs with <sup>51</sup>Cr (Perkin-Elmer) and then incubated the APCs with different drugs and TCLs or T-cell clones with an effector cells/target cells ratio of 5:1 to 40:1.<sup>22</sup> For the antibody blockage assay, autologous B-LCLs were pretreated with HLA class I antibody (W6/32; BioLegend, San Diego, Calif) or class II antibody (IWA12). For pulsing experiments, the irradiated autologous B-LCLs were first pulsed with the drugs overnight, and then the drugs were washed away before performing T-cell cytotoxicity assays.

**Surface plasmon resonance measurement and analyses**

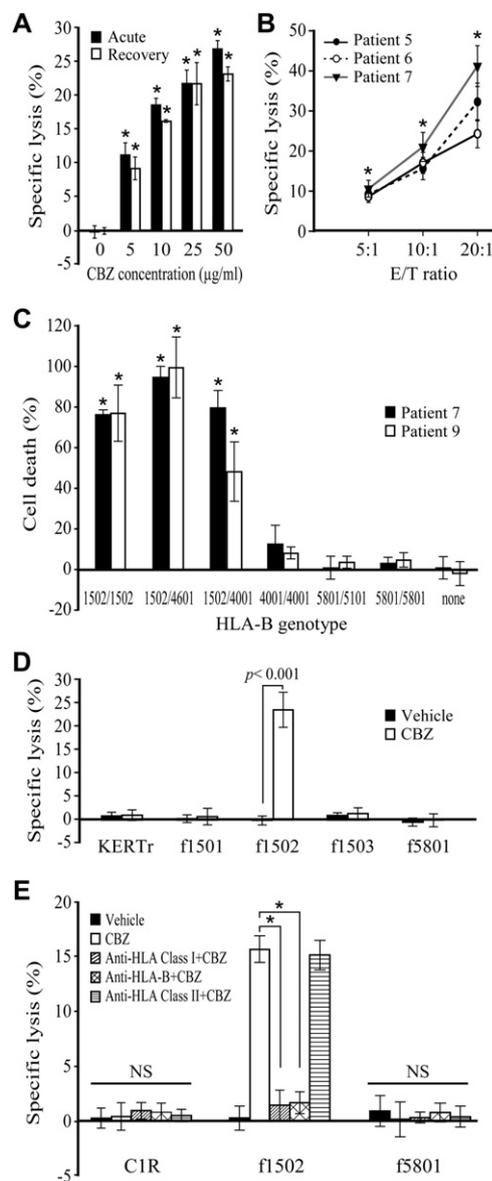
Complexes of the soluble recombinant proteins HLA-B, endogenous peptide, and β<sub>2</sub>-microglobulin were purified from the culture medium of C1R transfectants by using an affinity column coated with w6/32 antibody, as previously described.<sup>23</sup> We used the Biacore T100 surface plasmon resonance (SPR) biosensor (GE Healthcare, Piscataway, NJ) for analyzing the interaction between HLA-B proteins and drugs (see Fig E1). Briefly, we immobilized the purified soluble HLA-B proteins (acting as ligands) on the chips by means of an amine-coupling reaction, and the immobilized levels of sHLA-Bs were 9373-9812 response units. The drugs dissolved in PBS with 5% dimethyl sulfoxide were used for analysis and flowed through the solid phase. Responses of the interaction were reference subtracted and corrected with a standard curve for the dimethyl sulfoxide effects. We used BIA Evaluation Version 3.1 for data analysis.

**HLA-B\*1502 peptide-binding assay**

We used 16 synthetic peptides (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) to investigate the CBZ/peptide/HLA-B\*1502 interaction based on CTL cytotoxic activity. A C1R-f1502 stable clone was generated by means of transfection of the full-length cDNA plasmid encoding HLA-B\*1502.<sup>23</sup> The endogenous peptides in HLA-B\*1502 were removed by using cold mild citric acid.<sup>24</sup> After neutralizing with cultured medium, the cells were incubated with β<sub>2</sub>-microglobulin (4 µg/mL), GolgiStop (1 µL/mL; BD Biosciences, San Jose, Calif), and the synthetic peptides, respectively, for 3 hours at room temperature. The reconstitution was detected by using anti-HLA class I antibody (w6/32) with flow cytometry.

**In silico modeling of drugs and the peptide-loaded HLA-B\*1502 complex**

A homology protein model of peptide-loaded HLA-B\*1502 (accession no. BAA08824) was constructed by using HLA-B\*1501 protein (PDB ID: 1XR8) as the template structure. The candidate chemical binding sites were predicted by using the DS::MCSS and DS::Flexible Docking protocol (Discovery Studio version 2.5). Methods describing detailed information of computer modeling can be found in the Methods section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).



**FIG 1.** HLA-B\*1502-dependent activation of CBZ-specific CTLs in patients with CBZ-induced SJS/TEN. **A** and **B**, *In vitro*-expanded CTL TCLs displayed specific lysis against autologous B-LCLs in a CBZ concentration- and effector cells/target cells ratio-dependent manner. **C**, CBZ-specific CTL TCLs induced B-LCL cell death, as measured by means of annexin-V labeling in flow cytometry. HLA-B genotypes of B-LCLs are shown on the *horizontal axis*. **D** and **E**, Cytotoxicity of CBZ-specific T-cell clones against different HLA-B transfectants in the absence (**D**) or presence (**E**) of antibodies. \* $P < .01$ . NS, Not significant. Data of Fig 1, **A**, **B**, **D**, and **E**, were obtained by using the  $^{51}\text{Cr}$ -releasing assay. Results are representative of 3 independent experiments (means  $\pm$  SDs).

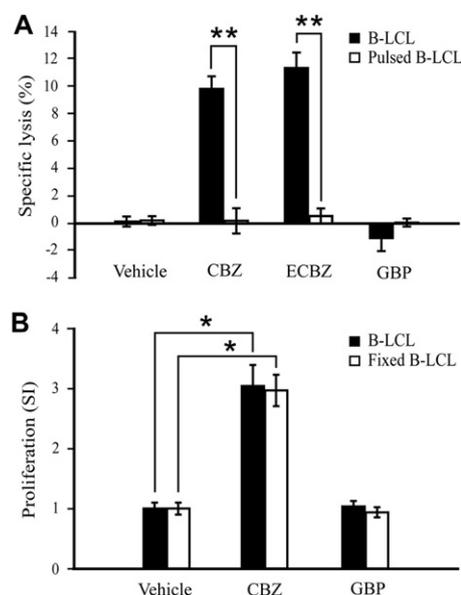
### Statistical analysis

All  $P$  values were calculated with the 2-tailed Student  $t$  test.

## RESULTS

### HLA-B\*1502-dependent activation of CBZ-specific CTLs in patients with CBZ-induced SJS/TEN

We performed *in vitro* culture and found that after approximately 4 to 5 cycles of coculture, the major population of CBZ-enriched TCLs was  $\text{CD8}^+$  (see Table E1). We detected the

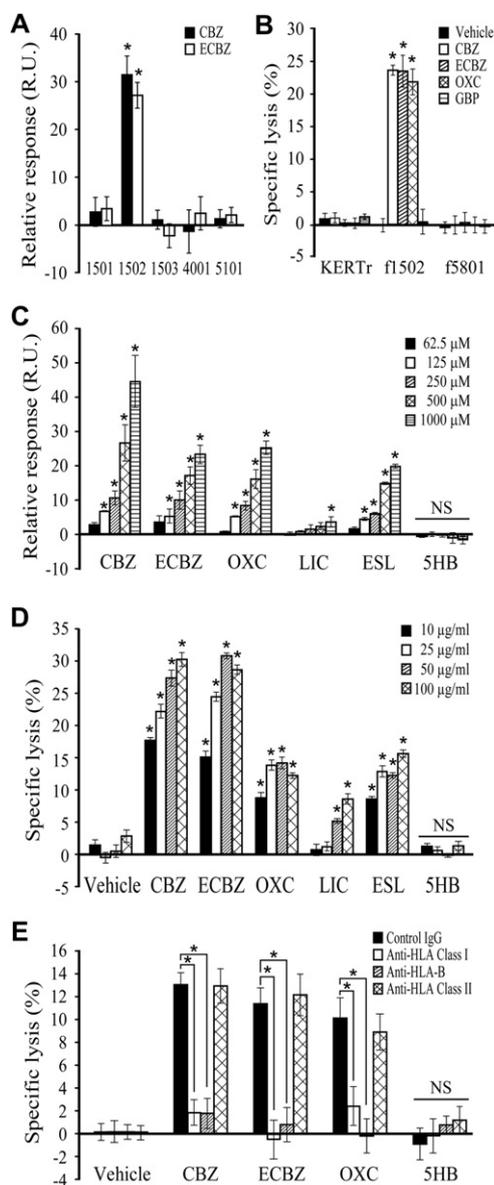


**FIG 2.** No involvement of intracellular metabolism or antigen processing in the CTL activation in patients with CBZ-induced SJS/TEN. **A**, CTL TCLs failed to kill CBZ- or ECBZ-pulsed autologous B-LCLs. Autologous B-LCLs pretreated with or without drugs at 37°C overnight and washed extensively are shown as *Pulsed B-LCL* ( $n = 5$ ). **B**, CBZ-specific CTL TCL proliferation against prefixed or nonfixed APCs ( $n = 3$ ). \* $P < .05$ ; \*\* $P < .001$ . GBP, Gabapentin; SI, stimulation index.

CBZ-specific CTL response in all of the 20 patients with CBZ-induced SJS/TEN, but it was absent in the 4 CBZ-tolerant control subjects. A total of 12 CBZ-specific T-cell clones with CD8 markers were obtained from the TCLs of 3 patients, although these clones could not be maintained *in vitro* for more than 2 months. CBZ-specific cytotoxicity was detected in a CBZ concentration-dependent manner and increased with the enlargement of effector cells/target cells ratios (Fig 1, **A** and **B**). Furthermore, CTLs exhibited cytotoxicity against B-LCLs or keratinocyte transfectant expressing the HLA-B\*1502 allele (Fig 1, **C** and **D**), which could be blocked by either anti-HLA class I or anti-HLA-B antibodies but not by anti-HLA class II antibodies (Fig 1, **E**). These data revealed that CBZ-specific CTLs were activated in an HLA-B\*1502-dependent manner.

### HLA-B\*1502 protein directly presents CBZ without intracellular metabolism or antigen processing

We evaluated antigen presentation in patients with CBZ-induced SJS/TEN using drug pulsing and APC-fixing assays. The cytotoxicity elicited by CBZ or its metabolite, ECBZ, could be abolished by a washing procedure (Fig 2, **A**). Fixing the APCs with paraformaldehyde could not destroy CBZ-specific TCL proliferation (Fig 2, **B**). These data indicated that the interaction between CBZ (or its metabolite) and HLA-B\*1502 was labile, and there was no involvement of the intracellular metabolism or antigen processing in patients with CBZ-induced SJS/TEN. Furthermore, HLA-B\*1502 directly bound CBZ and ECBZ, as detected by using SPR assays; however, other HLA-B recombinant proteins could not (Fig 3, **A**). The HLA-B\*1502-dependent CTL cytotoxicity could be detected when the culture medium contained CBZ, ECBZ, or OXC (Fig 3, **B**). These results indicated that HLA-B\*1502 protein specifically and directly binds



**FIG 3.** The interaction between HLA-B\*1502 protein and CBZ derivatives. **A**, Binding responses among 5 soluble HLA-B molecules and CBZ/ECBZ (500  $\mu\text{mol/L}$ ). **B**, Cytotoxicity of CTL TCLs against HLA-B transfectants and CBZ structure-related chemicals. **C**, Binding responses of HLA-B\*1502 recombinant protein toward CBZ-related compounds. **D** and **E**, Cytotoxicity of CTL TCLs against HLA-B\*1502 transfectants and CBZ-related compounds in the absence (Fig 3, **D**) or presence (Fig 3, **E**) of antibodies.  $*P < .01$ . NS, Not significant (Student *t* test). Data of Fig 3, **B**, **D**, and **E**, were obtained by using the  $^{51}\text{Cr}$ -releasing assay. Results are representative of 3 (Fig 3, **B**, **D**, and **E**) or 6 (Fig 3, **A** and **C**) independent experiments (means  $\pm$  SDs).

to CBZ or its derivatives and then activates CTLs in patients with SJS/TEN.

### The 5-carboxamide chemical moiety of CBZ is required for HLA-B\*1502 presentation

We analyzed the binding ability of HLA-B\*1502 recombinant protein toward compounds with a tricyclic ring structure like that of CBZ, including ECBZ, OXC, ESL, LIC, and 5HB (see Fig E1). The binding response of recombinant HLA-B\*1502 protein to CBZ analogs increased in a drug concentration-dependent

manner, with an estimated binding affinity of the micromolar range (Fig 3, **C**). In addition, ECBZ, OXC, and ESL also showed binding affinity, although the responses were weaker than those of CBZ (Fig 3, **C**). A very weak binding of LIC toward HLA-B\*1502 was detected only when LIC was present at a very high concentration (1000  $\mu\text{mol/L}$ ; Fig 3, **C**). No binding response was detected when using 5HB (Fig 3, **C**). A similar pattern of CTL cytotoxicity was detected when using these drugs as antigens (Fig 3, **D**). The cytotoxicity elicited by CBZ, ECBZ, or OXC could be blocked by either anti-HLA class I or anti-HLA-B antibodies but not by anti-HLA class II antibody (Fig 3, **E**).

### HLA-B75 family members exhibit binding ability to CBZ for antigen presentation in patients with CBZ-induced SJS/TEN

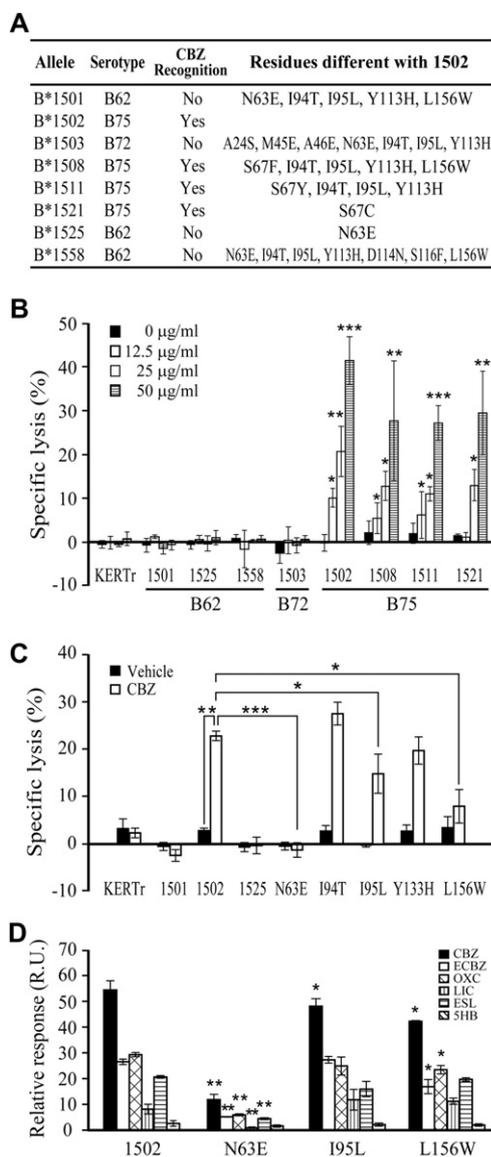
We examined the potential of CBZ binding and antigen presentation by HLA-B15 family members (Fig 4, **A**). The KERTr transfectants acquired susceptibility to CBZ-specific CTL cytotoxicity on the transfection of HLA-B75 family members, whereas no cytotoxicity was detected when expressing HLA-B62 or HLA-B72 family members (Fig 4, **B**). These results suggested that members of the HLA-B75 family share a similar conformation for CBZ binding and presentation and are capable of activating CTLs.

### Key residues in the peptide-binding groove of HLA-B\*1502 for CBZ presentation

We performed site-directed mutagenesis for HLA-B\*1502 and used HLA-B\*1501 as the control allele to pinpoint the CBZ binding site. We generated 5 site-directed mutagenic clones and stably transfected those into the KERTr cell line to examine CTL cytotoxicity (Fig 4, **C**). CBZ-specific lysis was attenuated when incubating CTLs with KERTr transfectants expressing I95L or L156W mutants and was completely abolished on incubation with the N63E mutant (Fig 4, **C**). We found consistent results from SPR analyses because I95L or L156W substitutions in HLA-B\*1502 recombinant protein led to the attenuation of binding to CBZ analogs and the N63E mutant resulted in a dramatic decrease in the binding reaction (Fig 4, **D**). These results suggested that residues 63N, 95I, and 156L of HLA-B\*1502 are required for CBZ presentation and T-cell activation. In particular, 63N shared by members of HLA-B75 was the key residue.

### Endogenous peptides loaded on HLA-B\*1502 proteins are required for CBZ presentation and CTL activation in patients with CBZ-induced SJS/TEN

To determine the involvement of specific peptides in the presentation of CBZ by HLA-B\*1502, we examined 12 HLA-B\*1502-binding peptides and 4 control peptides (see Table E2). In the presence of endogenous peptides, CBZ induced CTL cytotoxicity (Fig 5). After removing the heterogeneous endogenous binding peptides on HLA-B\*1502 by using acid treatment, CBZ-specific lysis was abolished (Fig 5). The specific lysis was reconstituted by loading APCs with respective HLA-B\*1502-binding peptide but not the control peptides (Fig 5). These results revealed that the presentation of CBZ requires endogenous peptides loaded in the antigen-binding groove of HLA-B\*1502 protein.



**FIG 4.** Key residues of HLA-B75 family members are involved in CBZ presentation and CTL activation. **A**, Summary of different residues and the ability of CBZ recognition of 8 HLA-B\*15 family members. **B** and **C**, Cytotoxicity of CTL TCLs against CBZ and KERTr transfectants expressing different HLA-B alleles and HLA-B\*1502 mutants ( $n = 5$ ). **D**, Binding responses of HLA-B\*1502 mutant proteins toward CBZ-related compounds ( $n = 10$ ). \* $P < .01$ ; \*\* $P < .05$ ; \*\*\* $P < .001$ .

### ***In silico* modeling highlights the molecular map of CBZ in HLA-B\*1502**

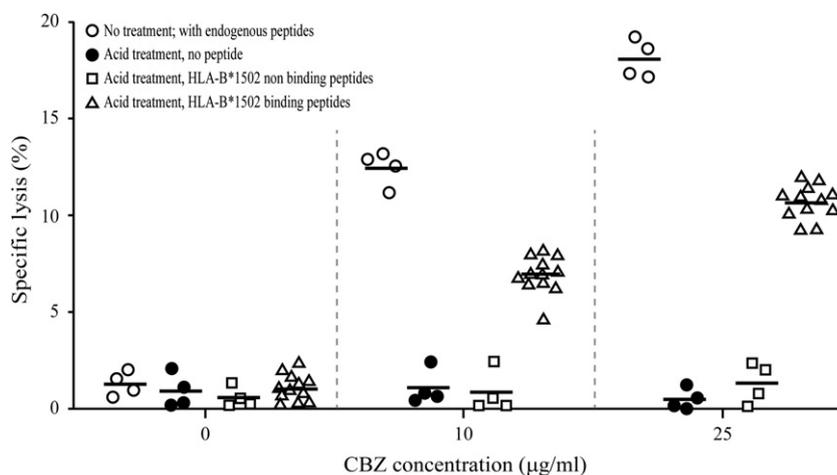
We used HLA-B\*1501 protein (PDB ID: 1XR8) as the template structure to construct the homologous protein model of the peptide (pp5, FLFDGSPTY)–loaded HLA-B\*1502 complex. Most of the best minimal multiple copy simultaneous search (MCSS) scores for CBZ were in the B pocket of the HLA-B\*1502 protein, which is close to the p2/p3 residues of pp5 (see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). We used the DS::CDOCKER module to dock CBZ and the AED compounds into the B pocket, and we consistently observed CBZ and ECBZ in the binding groove near Arg62 (Fig 6, A). Most energetically favorable drug-binding positions were predicted in the B pocket (see Fig E2), and the strongest binding sites were located

at Arg62 (Fig 6, A). Interestingly, the side chain of Arg62 could form a hydrogen bond with the ketone group of 5-carboxamide of CBZ, and the B pocket of HLA-B\*1502 was well fit by the tricyclic group of CBZ (Fig 6, B). By comparison, LIC, which has a hydroxyl group replacing the hydrogen atom at position 10 of the tricyclic ring of CBZ, cannot form a good fit into the B pocket. The binding energies of CBZ and LIC to the HLA-B\*1502/pp5 complex were  $-37.104$  kcal/mol and  $-19.252$  kcal/mol, respectively (Fig 6, B). The guanidine group of Arg62 on HLA-B\*1501 might be occupied by the carboxylate group of Glu63 (ie, salt bridge). By comparison, Arg62 of HLA-B\*1502 does not favor forming a bond with Asn63 (Fig 6, C). These results suggested that CBZ might interact with Arg62 of the B pocket of the HLA-B\*1502 protein and that Asn63 contributes to the specificity in molecular recognition.

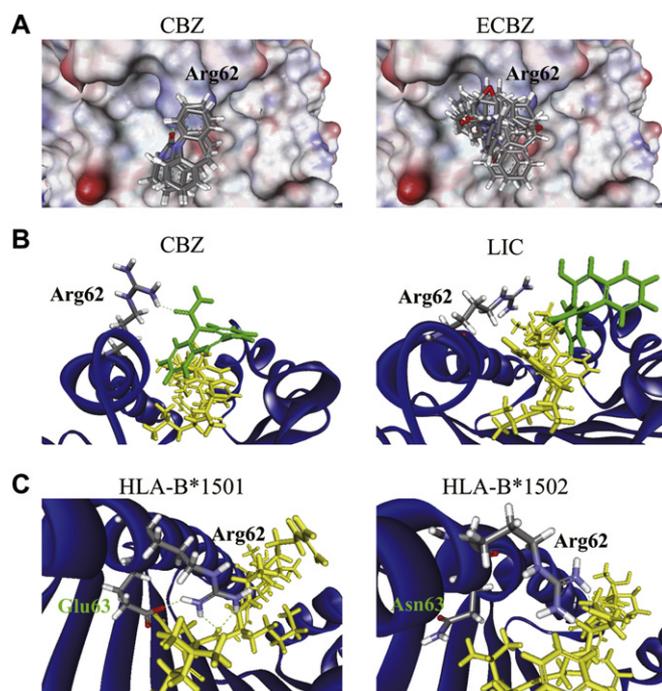
### **DISCUSSION**

In this study we obtained CBZ-specific TCLs, predominately CTLs after approximately 4 to 5 rounds of *in vitro* culture. The data suggested that the T-cell response in patients with SJS/TEN is very different from that seen in patients with maculopapular eruption or HSS. The lymphocyte transformation test (LTT) is frequently used for studying drug causality in patients with maculopapular eruption or HSS. However, LTT results are rarely positive in patients with SJS/TEN, even if performed by experienced allergy departments/laboratories.<sup>25</sup> By comparison, in this study we used TCLs for T-cell proliferation or CTLs for cytotoxicity assays and found 100% positivity (6 positive results in 6 examined TCLs) toward CBZ in the proliferation assays (data not shown). The discrepancy of data could be explained by the different materials of T cells: PBMCs in LTTs and TCLs in this study. Identification of drug causality by the primary T-cell proliferation in the LTT assay would be more difficult than using TCLs in <sup>51</sup>Cr-releasing assays for CTL-mediated SJS/TEN.

In the model of abacavir hypersensitivity, T cells respond to abacavir in subjects expressing HLA-B\*5701 through a tapasin- and processing-dependent pathway.<sup>10</sup> Furthermore, computer-based studies showed that abacavir binds to HLA-B\*5701 without peptide loading.<sup>26</sup> In contrast, in our model the presentation of CBZ by HLA-B\*1502 to CTLs does not require intracellular metabolism or processing yet does require endogenous peptide binding to stabilize the HLA class I complex on the cell surface. These data are consistent with our previous report of no detection of covalent bound CBZ in the heterogeneous HLA-B\*1502/peptide complex.<sup>23</sup> In addition, our modeling data also suggest that Arg62, which is located in the region around the B pocket, was the most probable binding site for drugs, and the interaction between the active side chain of Arg62 and 5-carboxamide on CBZ derivatives is controlled by the neighboring residues, such as Glu63 of HLA-B\*1501 or Asn63 of HLA-B\*1502. Our results support the p-i concept (direct pharmacologic interaction of a drug with immune receptors) that the interactions between the MHC and chemically inert drugs are labile.<sup>27-29</sup> However, our data could not exclude the possibility that CBZ metabolites bound irreversibly to HLA-B\*1502–restricted peptides might also stimulate T cells from patients with SJS. Further experiments are needed to clearly define the drug antigen that stimulates the response in patients, including the identification of relevant CBZ haptens and proteins with which CBZ metabolites interact in patients and generation of protein conjugates to study in T-cell assays.



**FIG 5.** Peptides loaded on HLA-B\*1502 are required for CBZ presentation and CTL activation. The influence of peptide on the presentation of CBZ by HLA-B\*1502 was evaluated by using acid treatment, peptide loading, and cytotoxicity assay by  $^{51}\text{Cr}$ -release. A total of 16 peptides, including 12 HLA-B\*1502-binding peptides and 4 non-HLA-B\*1502-binding peptides (listed in Table E2) were examined, and symbols represent each peptide in 4 independent experiments. Small horizontal lines indicate means.



**FIG 6.** Computer modeling of the molecular interaction between HLA-B\*1502 and CBZ and its derivatives. **A**, Simulation of the best conformation for drug/HLA-B\*1502/peptide complex in the B pocket using the DS::CDOCKER module. CBZ (left) and ECBZ (right) were identified near Arg62 in the binding groove of HLA-B\*1502. **B**, Prediction of the interaction between HLA-B\*1502 and chemicals (left, CBZ; right, LIC) by using the Flexible Docking program. The green dashed line represents the hydrogen bond between 5-carboxamide of CBZ and Arg62 of HLA-B\*1502. HLA-B\*1502, PP5 peptide, and chemicals are shown in blue, yellow, and green, respectively. **C**, The ionic interaction between Arg62 and Glu63 of HLA-B\*1501 (left) occupied the functional group for CBZ binding. In comparison, Asn63 of HLA-B\*1502 (right) could not form stable binding with Arg62.

We detected the CBZ-specific CTL response not only in all of the patients with CBZ-induced SJS/TEN but also in 3 of 7 HLA-B\*1502-positive healthy volunteers, whereas this response was

absent in all of the CBZ-tolerant control subjects.<sup>22</sup> In addition, we have identified the shared and restricted TCR use in patients with CBZ-induced SJS/TEN.<sup>22</sup> The CBZ-induced SJS/TEN-specific TCR clonotypes VB-11-ISGSY and VB-11-GLAGVDN could be detected in the PBMCs of 6 of 29 HLA-B\*1502-positive healthy volunteers, whereas they were absent in all 11 tolerant patients.<sup>22</sup> These data are different from the report of Chessman et al,<sup>10</sup> in which abacavir responses were detected in “all” unexposed volunteers expressing HLA-B\*5701. The discrepancy suggests that the elicitation of a T-cell response in patients with CBZ-induced SJS/TEN requires other cofactors (eg, TCR clonotypes) in addition to HLA-B\*1502. Although this study using circulating CBZ-specific T cells could not fully represent the pathogenetic mechanism of cutaneous T cells in patients with SJS/TEN, the circulating T cells might share similar characteristics with cutaneous T cells, including the CD8/cutaneous lymphocyte antigen phenotype, cytotoxic proteins/granulysin expression, and TCR use.<sup>2,3,22,30,31</sup> Our results suggest that peptide-loaded HLA-B\*1502, 5-carboxamide of CBZ, and specific TCR clonotypes form an immune synapse for the initiation of robust immune reaction in patients with CBZ-induced SJS/TEN.

Our data clearly showed that the 5-carboxamide group is critical for the interaction of CBZ with HLA-B\*1502 and the stimulation of T cells. However, very subtle changes in the ring structure of CBZ, such as OXC, LIC, and ESL, altered binding with HLA-B\*1502 and the T-cell response. These data are consistent with previous studies that show a lack of 5-carboxamide on CBZ or that a substitution of the amino group of 5-carboxamide attenuated the activation of CBZ-specific CD4<sup>+</sup> T cells in patients with CBZ-induced HSS.<sup>28,32</sup> OXC is considered a well-tolerated aromatic AED with a much lower incidence of SJS/TEN than CBZ.<sup>33,34</sup> OXC and ESL are rapidly and extensively metabolized to LIC in the human body.<sup>35,36</sup> In Taiwan SJS/TEN attributed to CBZ and OXC was estimated to be one in 2,000 and 10,000 exposures, respectively.<sup>37</sup> Because HLA-B\*1502 was also reported to be a common risk factor for OXC-induced SJS/TEN,<sup>38</sup> the lower incidence of OXC-induced SJS/TEN might be explained by the weaker binding ability of HLA-B\*1502 toward LIC.

In this study we used recombinant proteins and *in vitro*-expanded T cells to study the interaction between CBZ and HLA-B\*1502; however, these are limitations in this study to explain the complex immune reactions and great clinical diversity of patients. The drug hypersensitivity reactions in patients might be manifested by many cofactors, such as individual medical history, coadministered drugs, underlying diseases, viral infections, regulatory T-cell population/function, genetic polymorphisms of metabolic enzymes/immune-related genes, or autoimmune disorders.<sup>39,40</sup> Therefore the results presented in this study could not be extrapolated simply to the clinical situations of patients.

In conclusion, we have presented the first evidence, to our knowledge, of direct drug presentation by HLA molecules in patients with delayed-type drug hypersensitivity. Our *in vitro* system not only reveals that CBZ and its derivatives can directly interact with HLA-B\*1502 without cellular metabolism and antigen-processing presentation but also points out the key determinates for interaction on both chemicals and HLA-B75. The direct binding between HLA/peptide and a drug could elicit robust immune reactions in subjects who carry the specific HLA allele, and these results explained the pharmacogenetic linkage. This study not only provides new perspectives of the mechanisms of HLA-associated drug hypersensitivity but also sheds insight on the improvement of drug safety. A preclinic screen of the interaction between HLA and the pipeline chemicals could be useful for facilitating new drug development.

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#### Key messages

- HLA-B\*1502 and members of the HLA-B75 family directly interact with CBZ and its derivatives in patients with SJS and TEN.
- The endogenous peptide-loaded HLA-B\*1502 molecule presented CBZ to cytotoxic T cells without the involvement of intracellular drug metabolism or an antigen-processing pathway.
- HLA-B\*1502, peptides, CBZ, and TCRs of CTLs form an immune synapse to initiate the robust immune reactions in patients with CBZ-induced SJS/TEN.

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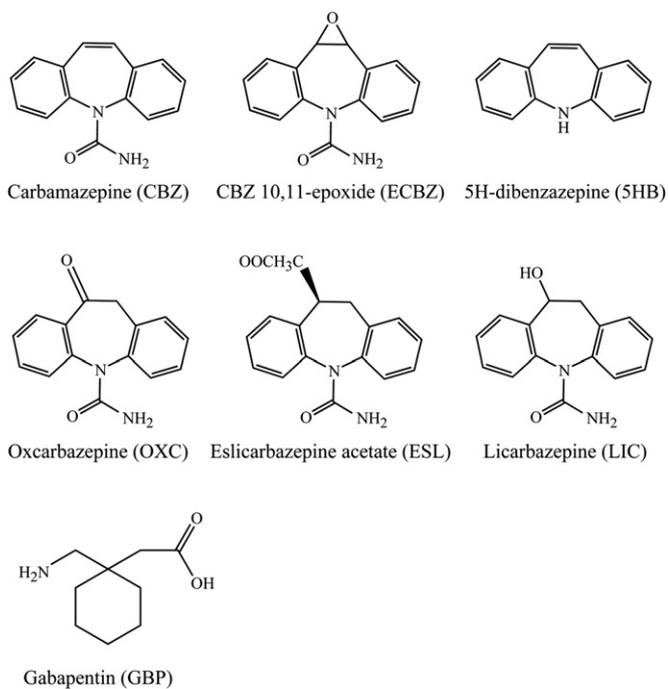
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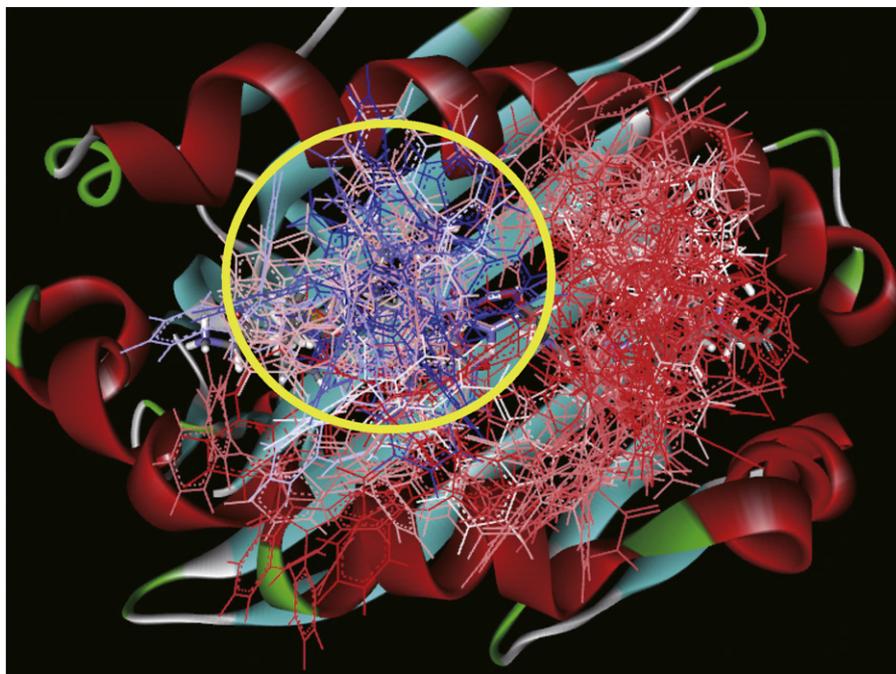
## METHODS

### ***In silico* modeling of drugs and the peptide-loaded HLA-B\*1502 complex**

We used *in silico* modeling to obtain a view of the spatial arrangements and interaction between drugs and the HLA-B\*1502/peptide complex. For construction of the peptide-loaded HLA-B\*1502 complex, FLFDGSPTY (pp5, Table E2) was selected and placed at the antigen-binding groove based on the coordinates of the x-ray peptide structure of HLA-B\*1501. The molecular dynamics simulation revealed the potential energy of the HLA-B\*1502/pp5 complex converged around 1000 ps. The molecular dynamics simulation after 3000 ps was further performed on the HLA-B\*1502/pp5 complex to obtain the

stable binding conformation. The MCSS was used to analyze the energetically favorable positions of the drugs within the groove of HLA-B\*1502 and scored the minima of these compounds within a 5-Å region of the peptide. We used the DS::CDOCKER module (CHARMm-Based Docking) and the DS::Flexible Docking protocol to dock the compounds into the binding pocket for refined poses of the compounds. Amino acids near Arg62 (including Arg62, Asn63, and sequence number approximately 1-4 of the peptide) were selected to have side-chain conformation change when performing docking. We examined the B pockets of both HLA-B\*1502 and HLA-B\*1501 proteins and found that the guanidium group of Arg62 of HLA-B\*1501 could form an electrostatic interaction with the carboxylate group of Glu63 (ie, salt bridge), which disrupts the interaction between Arg62 and CBZ.

**FIG E1.** Chemical structures of AEDs used in this study.



**FIG E2.** Predictive conformation of the interaction between CBZ and peptide/HLA-B\*1502 protein as modeled by using the multiple copy simultaneous search program (MCSS). CBZ within 5 Å of peptide was selected for analysis and colored by predicted binding magnitude (MCSS score), which represents the interaction energy between the compound and protein/peptide complex. The binding magnitude from strong to weak is colored from *blue* to *red*, and the hot zones of each simulation are circled in *yellow*.

**TABLE E1.** Clinical details of patients with CBZ-induced SJS/TEN and CBZ-tolerant donors

ID no.	Sex/age (y)	Time of drug exposure (y/mo/d)	Blood obtained since the episode of SJS/TEN (y)	Disease	Status	HLA-B	CD8 (%)*
1	F/38	10 d	2 y	SJS	Recovery	1502/4601	83
2	F/58	10 d	3 y	SJS	Recovery	1502/5101	85
3	M/22	21 d	3 y	SJS	Recovery	1502/3802	75
4	M/66	12 d	2 y	SJS	Recovery	1502/5101	89
5	F/33	10 d	3 y	SJS	Recovery	1502/5401	81
6	M/44	13 d	0 y	SJS	Active	1502/1302	76
7	M/26	14 d	1 y	SJS	Recovery	1502/1502	90
8	M/34	14 d	3 y	Overlapping SJS/TEN	Recovery	1502/4601	90
9	M/64	11 d	0 y	SJS	Active	1502/4601	75
10	M/53	ND	0.5 y	SJS	Recovery	1502/1502	93
11	F/15	16 d	2 y	SJS	Recovery	1502/4001	92
12	M/11	39 d	0 y	SJS	Active	1502/1302	92
13	M/73	23 d	0 y	SJS	Active	1502/1301	80
14	M/61	12 d	3 y	SJS	Recovery	1502/1502	88
15	F/80	12 d	1 y	SJS	Recovery	1502/5801	92
16	M/53	16 d	4 y	SJS	Recovery	1502/8101	77
17	F/19	13 d	1 y	SJS	Recovery	1502/1518	91
18	M/44	ND	0 y	SJS	Active	1502/1301	82
19	F/14	18 d	5 y	SJS	Recovery	1502/4001	95
20	F/23	13 d	0 y	SJS	Active	1502/0702	87
21	F/30	9 y	NA	Tolerant	NA	1502/4001	0
22	F/9	1 y	NA	Tolerant	NA	1502/5101	0
23	M/79	9 y	NA	Tolerant	NA	1502/4601	0
24	M/38	16 y	NA	Tolerant	NA	1502/1502	0

F, Female; M, male; NA, not applicable; ND, not determined.

\*The expression of CD8 on the TCLs was analyzed after approximately 4 to 5 cycles of *in vitro* culture.

**TABLE E2.** Peptide sequence

ID no.	Peptide sequence	Bind to HLA-B*1502
pp1	ELWKNPTAF	Yes
pp2	SLFVSNHAY	Yes
pp3	NVIRDAVTY	Yes
pp4	QLGPVGGVF	Yes
pp5	FLFDGSPTY	Yes
pp6	SQFGGGSQY	Yes
pp7	YLYGQTTY	Yes
pp8	FPYGTTVTY	Yes
pp9	YMIDPSGVSY	Yes
pp10	EQYEQILAF	Yes
pp11	SVKPASSSF	Yes
pp12	TILDSSSSF	Yes
np1	FLPSDFFPSV	No
np2	FVPPPPPP	No
np3	APPPPPPP	No
np4	APPPPPGH	No

*np*, Peptides that cannot be presented by HLA-B\*1502; *pp*, peptides that can be presented by HLA-B\*1502.