

Shared and restricted T-cell receptor use is crucial for carbamazepine-induced Stevens-Johnson syndrome

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Background: Stevens-Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), are life-threatening drug hypersensitivities with robust immune responses to drugs. Despite the strong HLA predisposition to drug hypersensitivities, such as HLA-B*1502 to carbamazepine (CBZ)-induced SJS/TEN, it remains unknown whether particular T-cell receptors (TCRs) participate in recognition of small drug/peptide-HLA complexes.

Objective: Using the strong HLA predisposition in patients with CBZ-induced SJS/TEN as a model, we aimed to study the use of TCR repertoire in patients with drug hypersensitivity.

Method: We enrolled patients with CBZ-SJS/TEN, tolerant control subjects, and healthy subjects who had no history of CBZ exposure. We isolated PBMCs from the subjects, cultured CBZ-specific T cells, and globally investigated the expression level and third complementarity-determining region length distribution of the TCR profile. We further assessed the pathogenic role of the disease-specific clonotype using real-time PCR-based tests and functional analysis.

Results: On drug stimulation, CBZ-specific CD8⁺ T cells were expanded *in vitro* and activated to release granulysin. Notably, VB-11-ISGSY was identified as the most predominant clonotype and shared among different subjects. This clonotype was present in 16 (84%) of 19 patients with SJS/TEN, absent in all 17 tolerant patients, and present at a low frequency in healthy subjects (4/29 [14%]). CBZ-specific cytotoxicity could be primed *in vitro* in the PBMCs of healthy subjects who are carriers of HLA-B*1502 and VB-11-ISGSY; this cytotoxicity could be

blocked by an anti-TCR-VB-11 antibody. Furthermore, a single T-cell clone expressing VA-22-FISGTY/VB-11-ISGSY showed significant cytotoxicity against HLA-B*1502-positive antigen-presenting cells and CBZ.

Conclusion: This study establishes the key role of the TCR in the pathogenic mechanism of SJS/TEN, explains why some HLA-B*1502 carriers are tolerant to CBZ, and provides a biomarker profile for drug hypersensitivity. (J Allergy Clin Immunol 2011;■■■■:■■■-■■■.)

Key words: Drug hypersensitivity, T-cell receptor, HLA, third complementarity-determining region, Stevens-Johnson syndrome, toxic epidermal necrolysis

Drug hypersensitivity remains a major clinical problem. Among the many types of drug hypersensitivity, Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and hypersensitivity syndrome (HSS) are 3 of the most serious and life-threatening conditions.¹ The pathogenesis of these serious drug hypersensitivities is not completely understood. Available evidence suggests that drug hypersensitivity is mediated by adaptive immunity, which involves MHC-restricted drug presentation, activation, and clonal expansion of T cells.²⁻⁴ Histopathological and immunologic findings revealed that SJS and TEN are predominately mediated by CD8⁺ cytotoxic T lymphocytes (CTLs) and natural killer T cells; these cells infiltrate the skin lesions and secrete granulysin and other cytotoxic proteins/chemokines, resulting in disseminated keratinocyte death in patients with SJS and TEN.⁵⁻⁷ However, because of the diversity of MHC polymorphisms, the enormous complexity of culprit drugs and their metabolites, the indeterminacy of the associated or bound peptides, and the difficulties with patient enrollment, little is known regarding how the diversity of T-cell receptor (TCR) repertoires is used and whether particular TCRs participate in the recognition of small drug compounds presented by HLA in T cell-mediated drug hypersensitivity.

The hapten hypothesis and the p-i concept (pharmacologic interaction with immune receptors) might explain how a small drug induces an immune reaction; both hypotheses propose that drug hypersensitivity involves the interactions between specific MHC molecules, TCRs, and drug/peptides.^{4,8} The exact MHC molecules involved in drug hypersensitivity were uncertain until the recent findings of strong genetic associations between HLA alleles and the reactions to specific drugs; examples are the associations of HLA-B*1502 with carbamazepine (CBZ)-induced SJS/TEN (CBZ-SJS/TEN), HLA-B*5801 with allopurinol-induced HSS/SJS/TEN, HLA-B*5701 with abacavir-induced HSS or with flucloxacillin-induced liver injury, and HLA class

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Abbreviations used

B-LCL:	B-lymphoblastoid cell lines
CA:	α -Chain constant region
CB:	β -Chain constant region
CBZ:	Carbamazepine
CDR3:	Third complementarity-determining region
⁵¹ Cr:	Chromium 51
CTL:	Cytotoxic T lymphocyte
GBP:	Gabapentin
HSS:	Hypersensitivity syndrome
OXC:	Oxcarbazepine
SJS:	Stevens-Johnson syndrome
TCR:	T-cell receptor
TEN:	Toxic epidermal necrolysis
VA:	α -Chain variable region
VB:	β -Chain variable region

II alleles with lumiracoxib-related liver injury.⁹⁻¹³ In particular, the association between HLA-B*1502 and CBZ-SJS/TEN is the strongest in Han Chinese¹⁴ and has been validated in patients from Hong Kong, Singapore, Thailand, Malaysia, and India, where this allele is prevalent.¹⁵⁻¹⁷ However, not all subjects carrying the HLA-B*1502 allele have SJS or TEN while taking CBZ. This suggests that some other factor or factors are involved in the T cell-mediated immune reactions that might contribute to CBZ-SJS/TEN.

Global analysis of the TCR repertoire by means of spectratyping the third complementarity-determining region (CDR3) of T cells could offer key information for understanding the effect of HLA polymorphism, heterogeneous inciting antigen, and tolerance on TCR use.¹⁸⁻²¹ The clonotypic analysis could measure disease-associated T cells in the human subjects, evaluate the expression profile of pathogenic T cells, and determine the correlation between TCR use and disease severity.²² Using the strong HLA predisposition in patients with CBZ-SJS/TEN as a model, we hypothesized that the effector T cells of patients with CBZ-SJS/TEN share a conserved TCR use against the small drug/peptide-HLA-B*1502 complex. In this study we characterized the TCR uses of patients with CBZ-SJS/TEN and compared these with the TCR uses of drug-tolerant control subjects.

METHODS**Patients and samples**

We enrolled subjects from Chang Gung Memorial Hospital Health System in Taiwan, including 18 patients with CBZ-SJS/TEN (17 patients with CBZ-SJS and 1 patient with CBZ-overlapping SJS and TEN, all of whom were HLA-B*1502 positive), 11 CBZ-tolerant control subjects (2 who were HLA-B*1502 positive and 9 who were HLA-B*1502 negative) who had been administered CBZ for at least 3 months without any cutaneous adverse reactions, and 29 HLA-B*1502-positive healthy volunteers who had no history of CBZ exposure. Because it has been difficult to enroll CBZ-tolerant control subjects who are HLA-B*1502 carriers because of the drug relabeling by the US Food and Drug Administration and because oxcarbazepine (OXC) is a 10-keto derivative of CBZ, we enrolled 1 patient with OXC-SJS and 6 OXC-tolerant control subjects who were all HLA-B*1502 carriers and had been administered OXC for at least 3 months without cutaneous adverse reactions.²³⁻²⁶ The clinical details of the HLA-B*1502-positive patients with SJS/TEN and tolerant control subjects are shown in Table E1 (available in this article's Online Repository at www.jacionline.org). Approval for the study was obtained from the institutional review board, and informed consent was obtained from each participant.

Culture of drug-specific T cells

PBMCs (2.0×10^6 cells) were cultured with CBZ (10 μ g/mL) and IL-2 (100 U/mL) in culture medium for 5 to 7 days, and then the cells were incubated with irradiated (50 Gy) EBV-transformed autologous B-lymphoblastoid cell lines (B-LCLs) or autologous PBMCs and CBZ (25 μ g/mL), as previously described.²⁴ According to the results generated with TCR transfectants, we used 100 μ mol/L (ie, approximately 25 μ g/mL) of CBZ for primary T-cell cultures. After 7 to 10 days of culture, proliferated T cells were recovered and subjected to repeat cycles of culture. At the end of the fifth cycle, T cells could be expanded to approximately 1.0 to 8.0×10^5 cells from the PBMCs of patients. By comparison, the T cells from the PBMCs of 8 drug-tolerant control subjects (2 CBZ-tolerant and 6 OXC-tolerant subjects) could not be *in vitro* maintained by CBZ stimulation for more than 2 cycles of coculture. Methods describing chemicals and generation of T-cell clones can be found in the **Methods** section in this article's Online Repository at www.jacionline.org.

Flow cytometry

We performed immunophenotypic analyses using distinct fluorochrome-conjugated mAbs that recognized human CD3 (UCHT1; BD Biosciences, Mississauga, Ontario, Canada), CD8 (RPA-T8, BD Biosciences), or TCR VB-11 (C21; Immunotech, Fullerton, Calif). The cells were examined by means of multicolor flow cytometry with a FACSCalibur (BD Biosciences), and data were analyzed with CellQuest acquisition software (BD Biosciences). The CD3⁺CD8⁺ T cells were isolated by means of FACS sorting on a BD FACSAria system (BD Biosciences).

ELISA

We determined the levels of IFN- γ and granulysin in T-cell culture supernatants using ELISA reagents (BD Biosciences), as described previously.⁵ The assay sensitivity for IFN- γ was 1 pg/mL, and that for granulysin was 0.625 ng/mL.

Determination of drug-specific cytotoxicity *in vitro*

We established C1R stable cell clones expressing the full-length HLA-B*1502 cDNA (C1R-B*1502 cells) or HLA-B*5801 cDNA (C1R-B*5801 cells), as previously described.^{8,27} We evaluated the cytotoxic specificity of drug-specific CTLs with a standard 4-hour chromium 51 (⁵¹Cr) release assay. Briefly, the target cells (1.0×10^6 cells) were labeled with ⁵¹Cr at room temperature for 1 hour. The target cells were washed 3 times with 50 mL of PBS to remove free ⁵¹Cr, and then 2.5×10^3 cells were incubated with T cells in 0.2 mL of culture medium with or without CBZ (25 μ g/mL). The effector/target ratios were as follows: 1:1, 3:1, 10:1, or 30:1. Specific lysis (as a percentage) was calculated as follows:

$$100\% \times (\text{Experimental release} - \text{Spontaneous release}) / (\text{Maximal release} - \text{Spontaneous release}).$$

Methods describing the analysis of drug-specific cytotoxicity can be found in the **Methods** section in this article's Online Repository.

TCR repertoire analysis

Total RNA of T cells was isolated and reverse transcribed into cDNA. The clonotypes of VA and VB were determined by means of PCR amplification with TCR β -chain constant region (CB)/TCR α -chain constant region (CA)-specific primers and 33 specific primers pairs against TCR α -chain variable region (VA) gene families and 25 specific primer pairs against β -chain variable region (VB) gene families (see Tables E2 and E3 in this article's Online Repository at www.jacionline.org), as previously described.²⁸ Methods describing the analysis can be found in the **Methods** section in this article's Online Repository.

Quantitative real-time PCR

We applied quantitative real-time PCR and VA/VB-specific primers to analyze the expression levels of the TCR VA or VB subfamilies in the

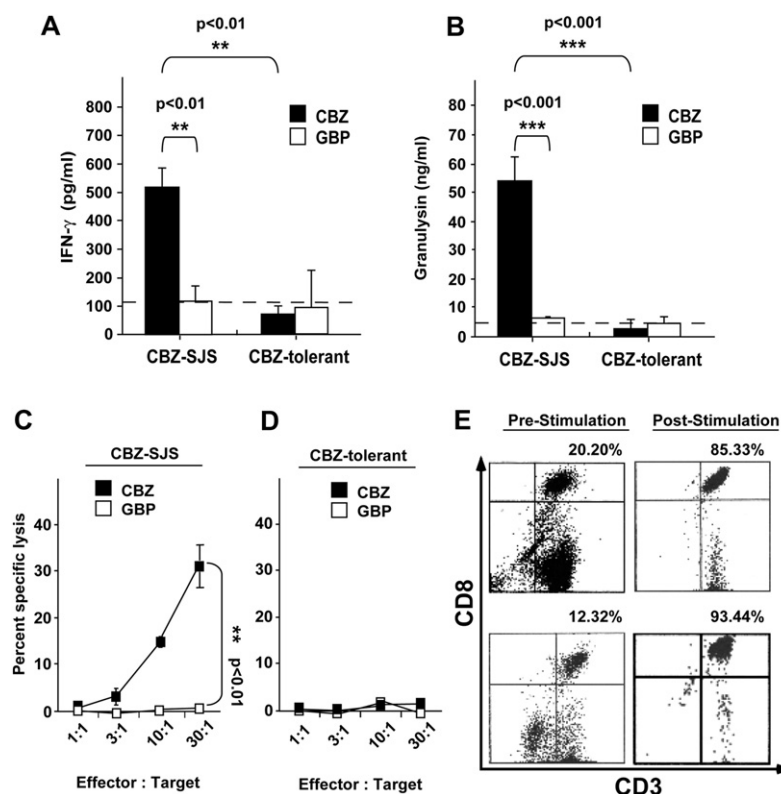


FIG 1. CBZ-specific T-cell responses of patients with CBZ-SJS and CBZ-tolerant control subjects. **A** and **B**, Levels of IFN- γ (Fig 1, A) and granulysin (Fig 1, B) were determined in the culture supernatants of CBZ-stimulated T cells (after 2 cycles of culture with CBZ) from patients ($n = 4$) and tolerant control subjects ($n = 2$). The dashed line indicates a mean level of IFN- γ or granulysin detected by means of ELISA in the T-cell culture supernatants in the absence of drug stimulation ($n = 8$). **C** and **D**, CBZ-specific T-cell (after 2 cycles of culture with CBZ) cytotoxicity was determined by using ^{51}Cr release assays. **E**, Flow cytometric immunophenotypic analysis of the CD8 $^{+}$ subsets in CBZ-stimulated T cells obtained from the fifth cycle for 2 representative patients with CBZ-SJS/TEN. Numbers indicate the percentage of cells in the CD3 $^{+}$ /CD8 $^{+}$ quadrant. $^{*}P < .01$ and $^{***}P < .001$, paired t test.

expanded T cells.²⁹ Primers in Tables E4 and E5 and methods describing the analyses of quantitative real-time PCR can be found in the Methods section in this article's Online Repository.

RESULTS

CBZ-specific T-cell responses in a conserved HLA-B*1502 genetic background

The drug-specific T-cell response was examined at the end of the second cycle of culture with CBZ. Compared with cells from tolerant control subjects, cells from patients with CBZ-SJS released higher levels of IFN- γ and granulysin (5- and 7-fold, respectively) on stimulation with CBZ, and these increases were drug specific because treatment with the control drug gabapentin (GBP) had no effect (Fig 1, A and B). These *in vitro* expanded T cells expressed CD3/CD8 markers and granulysin, which are characteristic of the blister cells found in the skin lesions of patients with SJS/TEN.⁵ Furthermore, T cells from patients with CBZ-SJS showed strong cytotoxic responses against autologous B-LCLs with an increase in effector/target cell ratios in the presence of CBZ but not GBP (Fig 1, C). By comparison, T cells from tolerant control subjects showed no cytotoxicity in the presence of CBZ or GBP (Fig 1, D). After 5 cycles of CBZ incubation, an enrichment of CD8 $^{+}$ T-cell subsets of up to 93% was observed from the PBMCs of patients with CBZ-SJS/TEN (Fig 1, E).

Common skewed TCR VB and VA repertoires of drug-specific CTLs

Common skewed and restricted VA and VB spectratyping profiles were found in the oligoclonal expansion of T cells of 8 patients with CBZ-SJS/TEN examined (Fig 2). As examples, the most skewed VB profile, VB-11, was found in all patients with CBZ-SJS/TEN (100% of patients); the VB-13B, VB-14, and VB-15 profiles were found in 5 of 8 patients; and the VB-4, VB-16, VB-20, and VB-24 profiles were found in 4 of 8 patients (Fig 2, A). Similar skewed spectratype profiles for VA-1B, VA-3, VA-12, VA-15, VA-22, VA-25, and VA-26 were found in the T cells of different patients (Fig 2, B). In particular, the skewed VA-22 profile was found in all patients. By comparison, T cells obtained from 8 drug-tolerant control subjects at the end of the second cycle exhibited Gaussian spectratype profiles, a hallmark pattern indicating polyclonal T-cell repertoire, and no expansion of antigen-specific T cells (Fig 2, C, and see Fig E1 in this article's Online Repository at www.jacionline.org).

Upregulation of skewed VB and VA subfamilies in patients with CBZ-SJS/TEN

Using quantitative real-time PCR, we found that the VB-11 subfamily of T cells from the PBMCs of all 8 patients with

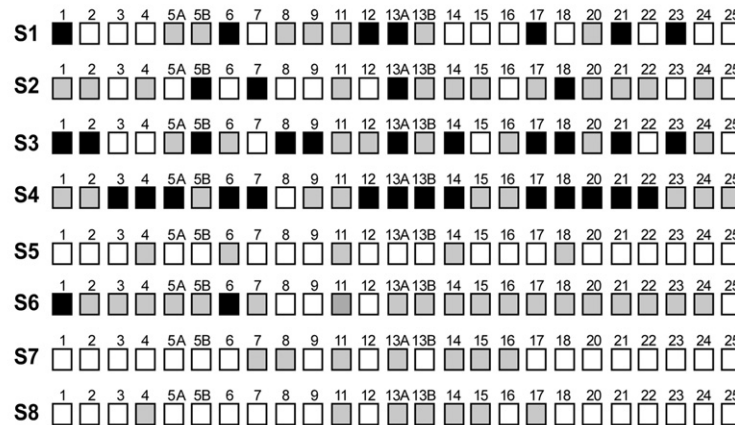
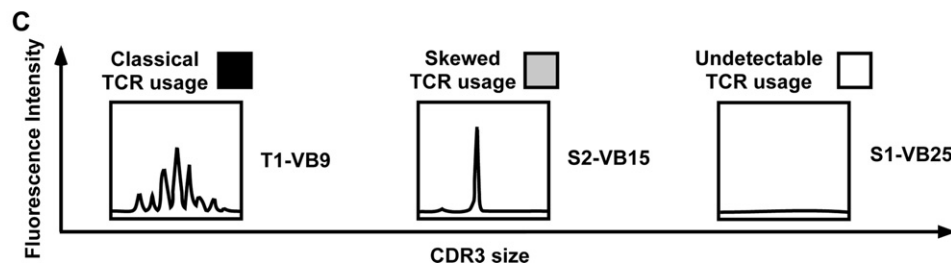
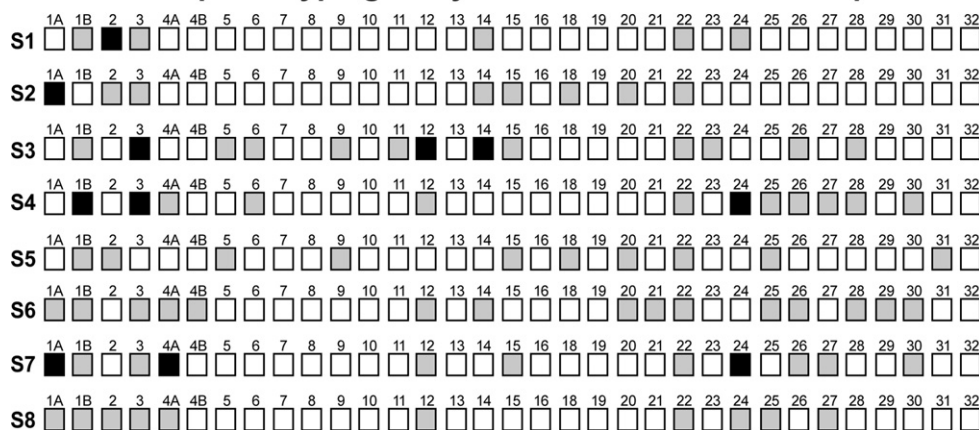
A CDR3 spectratyping analysis of VB in CBZ-SJS/TEN patients**B CDR3 spectratyping analysis of VA in CBZ-SJS/TEN patients**

FIG 2. Common skewed TCR VB and VA repertoires of CBZ-specific CTLs. **A** and **B**, CDR3 spectratype patterns of VB (Fig 2, A) and VA (Fig 2, B) of CBZ-stimulated CD8⁺ T cells obtained from the fifth cycle of *in vitro* cultures from 8 patients with CBZ-SJS/TEN. **C**, The x-axis displays CDR3 length, and the y-axis indicates fluorescence intensity of the runoff products of each TCR subfamily. **Black squares** indicate a normal CDR3 size profile (quasi-Gaussian distribution, >5 peaks), **gray squares** indicate a restricted CDR3 size profile (skewed TCR repertoire, 1-4 peaks), and **open squares** indicate an undetectable CDR3 size profile (undetectable TCR, 0 peak).

CBZ-SJS/TEN showed the highest expression on CBZ stimulation, with the expansion up to 26 ± 8.0 -fold compared with the CBZ-unstimulated T cells (Fig 3, A). Other TCR VB subfamilies with skewed spectratypes, including VB-5B and VB-15, also had an approximately 5- to 10-fold increase of expression (Fig 3, A). In addition, the skewed VA-4A, VA-12, and VA-22 subfamilies of TCRs were predominantly expanded with 4 ± 1.3 -, 11 ± 11.2 -, and 12 ± 3.6 -fold increases (Fig 3, B). Of note, 2 skewed TCR spectratypes, VB-11 and VA-22, showing the highest level of expression in each panel might pair with each other to form TCR and represent the clonal expansion of CBZ-specific T cells

on CBZ stimulation. In contrast, *in vitro* incubation of CBZ could not result in specific TCR VA or VB expansion from the T cells of PBMCs of 8 tolerant control subjects (2 CBZ-tolerant and 6 OXC-tolerant subjects; Fig 3).

CDR3 sequence analysis confirms the oligoclonality of CBZ-specific T cells

Because the skewed VB-11 and VA-22 subtypes were the most frequently expanded spectratypes of T cells from patients with CBZ-SJS/TEN, we performed clonotyping and CDR3 sequence

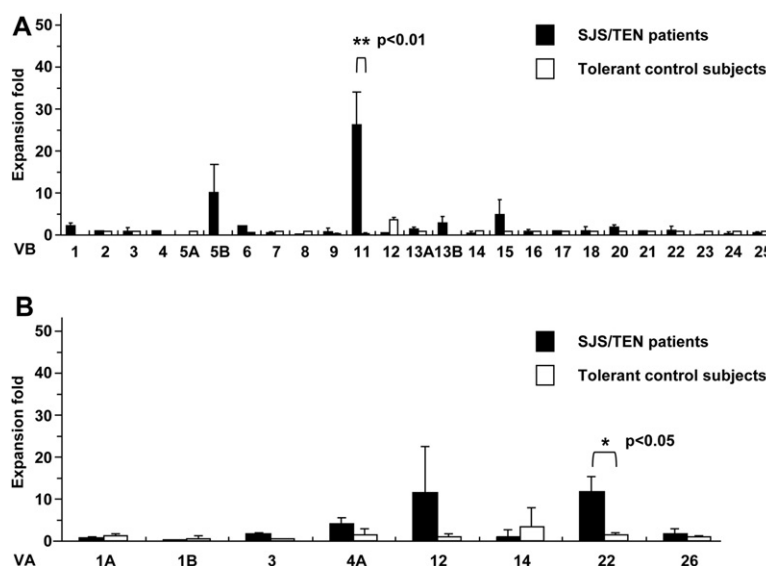


FIG 3. Upregulation of skewed VB and VA subfamilies in patients with CBZ-SJS/TEN. **A** and **B**, cDNA expression levels of TCR VB (**Fig 3, A**) and VA (**Fig 3, B**) subfamilies of 8 patients with SJS/TEN or 8 tolerant control subjects (2 CBZ-tolerant and 6 OXC-tolerant subjects) were measured by using quantitative real-time PCR and normalized by the levels of the TCR constant region (CB or CA). Bars indicate the expansion fold of TCR VB (**Fig 3, A**) and VA (**Fig 3, B**) in CBZ-stimulated T cells (after 2 cycles of culture with CBZ), with levels compared with those of T cells in the absence of CBZ stimulation. * $P < .05$ and ** $P < .01$, unpaired t test.

analysis for VB-11 and VA-22. Most of the TCR VB-11 and VA-22 clonotypes showed skewed patterns with only 1 peak or 2 to 3 peaks, strongly suggesting nonrandom oligoclonal expansion (exemplified by VB-11 for patients S1, S2, and S7 in **Fig E2** in this article's Online Repository at www.jacionline.org). The high frequencies of unique in-frame CDR3 sequences among the total analyzed TCR VB clonotypes for these 8 patients demonstrated the preferential use of the β -chain joining region of TRBJ2-1*01 in patients S1, S2, S3, S4, S5, and S7 and TRBJ2-7*01 in patients S4, S6, and S8 (**Table I**). Notably, the 2 most immunodominant CDR3 clonotypes, VB-11-ISGSY and VB-11-GLAGVDN, were shared among 5 and 2 of the 8 patients with CBZ-SJS/TEN, respectively (**Table I**). Furthermore, the CDR3 sequence analysis of the skewed VA profile revealed 3 immunodominant clonotypes, VA-22-FISGTY, VA-22-SEVIATFG, and VA-22-LSYNQGG, which were shared among 3, 2, and 2 of the 8 patients with CBZ-SJS/TEN, respectively (**Table I**).

Detection of immunodominant TCR clonotypes in the blister cells and PBMCs of patients with CBZ-SJS/TEN and their correlations with clinical stages

Two VB-11 clonotypes, VB-11-ISGSY and VB-11-GLAGVDN, were used as biomarkers to examine their presence and quantify their levels in blister cells and PBMCs of patients with CBZ-SJS/TEN at different disease stages. Both clonotypes could be detected in the PBMCs or blister cells of patients with CBZ-SJS/TEN, with expression levels of approximately 5- to 10-fold higher in PBMCs from patients during the active stage and approximately 14- to 17-fold higher in the blister fluid cells compared with the levels of PBMCs from patients during the recovery stage (**Fig 4, A**). By comparison, 2 randomly selected TCR clonotypes (VB-11-EYTPAAP and VB-11-RDPDT) from a CBZ-tolerant control subject and a healthy donor showed

undetectable expression (**Fig 4, A**). In addition, we found that the VB-11-ISGSY clonotype showed high levels of expression in PBMCs obtained during the disease recovery stage of 13 (93%) of 14 patients with CBZ-SJS/TEN (**Fig 4, B**). However, the VB-11-ISGSY clonotype was absent in all 11 CBZ-tolerant control subjects (2 HLA-B*1502-positive and 9 HLA-B*1502-negative subjects) and present at a low frequency in healthy subjects (4/29 [14%]; **Fig 4, B**). Another immunodominant clonotype, VB-11-GLAGVDN, was present in 5 (36%) of 14 patients with CBZ-SJS/TEN, absent in all 11 CBZ-tolerant control subjects, and present in only 2 (7%) of 29 healthy subjects (**Fig 4, C**). We also found the VB-11-ISGSY clonotype was present in 1 HLA-B*1502-positive patient with OXC-SJS but absent in all 6 OXC-tolerant control subjects. The absence of these clonotypes in the tolerant control subjects might explain why these subjects do not have SJS/TEN.

In vitro priming of disease-specific clonotypes in healthy donors and blockade of CBZ-specific cytotoxicity with an antibody against TCR VB-11

Incubation of CBZ with PBMCs of subjects S2, H1, H2, and H3 who had the VB-11-ISGSY clonotype resulted in significant release of granulysin into the culture media (range, 23-54 ng/mL) in the *in vitro* priming tests (**Fig 5, A**). By comparison, very low granulysin levels (<5 ng/mL) were detected from the culture media of PBMCs of subjects H4, H5, H6, H7, and T2 who did not carry the clonotype (**Fig 5, A**). In addition, the CBZ-expanded T cells obtained from the VB-11-ISGSY-positive donors (H1 and H2) and patients (S1 and S2) and exhibited significant cytotoxic responses against target cells expressing HLA-B*1502 (C1R-B*1502 cells), whereas we could not obtain CBZ-specific T cells from the VB-11-ISGSY-negative donors (H4 and H5) or tolerant control subjects (T1 and T2; **Fig 5, B and C**). Furthermore, the

TABLE I. Oligoclonality and amino acid sequences of VB-11 and VA-22 CDR3 clonotypes of CBZ-stimulated CD8⁺ T cells from patients with CBZ-SJS/TEN

Case no.	VB	TRBV	TRBD	TRBJ	CDR3	Frequency (%)
S1	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNEQFF	82
	B11	TRBV25-1*01	TRBD2*02	TRBJ2-1*01	CASSGLAGVDNNEQFF	18
S2	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNERFF	100
S3	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNEQFF	50
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNEQSF	42
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-5*01	CASSEYMGIQETQYF	8
S4	B11	TRBV25-1*01	TRBD2*02	TRBJ2-1*01	CASSGLAGVDNNEQFF	39
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-7*01	CASSLGYEQYF	22
	B11	TRBV25-1*01		TRBJ1-1*01	CASSDNTEAFF	11
S5	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNEQFF	100
S6	B11	TRBV25-1*01		TRBJ2-7*01	CASSAHEQYF	83
	B11	TRBV25-1*01	TRBD1*01	TRBJ1-2*01	CASSEWGEVGKGYTF	17
S7	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	CASSISGSYNEQFF	100
S8	B11	TRBV25-1*01	TRBD1*01	TRBJ2-7*01	CASSEDRSPYEQYF	100
Patient no.	VA	TRAV	TRAJ		CDR3	Frequency (%)
S1	A22	TRAV9-2*01	TRAJ48*01		CALSDRHFNGNEKLTF	38
	A22	TRAV9-2*01	TRAJ57*01		CASEVIATFGNEKLTF	38
	A22	TRAV9-2*01	TRAJ31*01		CALSDQDARLMF	9
S2	A22	TRAV9-2*01	TRAJ57*01		CASEVIATFGNEKLTF	29
	A22	TRAV9-2*01	TRAJ57*01		CALGGSEKLVF	29
	A22	TRAV9-2*01	TRAJ52*01		CALSSSAGGTSYGKLTf	14
S3	A22	TRAV9-2*01	TRAJ9*01		CALSEGTTGGFKTIF	44
	A22	TRAV9-2*01	TRAJ40*01		CAFISGTYKYIF	22
	A22	TRAV9-2*01	TRAJ52*01		CALSDYAGGTSYGKLTf	11
S4	A22	TRAV9-2*01	TRAJ37*01		CALSPYGSNGTGKLIF	33
	A22	TRAV9-2*01	TRAJ23*01		CALSYNQGGKLIF	33
	A22	TRAV9-2*01	TRAJ57*01		CALSGGTSYGKLTf	33
S5	A22	TRAV9-2*01	TRAJ40*01		CAFISGTYKYIF	100
S6	A22	TRAV9-2*01	TRAJ23*01		CALSYNQGGKLIF	50
	A22	TRAV9-2*01	TRAJ37*02		CALSPPSNTGKLIF	25
S7	A22	TRAV9-2*01	TRAJ13*02		CALSSSSGGYQKVTF	60
	A22	TRAV9-2*01	TRAJ40*01		CAFISGTYKYIF	20
S8	A22	TRAV9-2*01	TRAJ57*01		CALMLSQGGSEKLVF	100

The designations TRBV, TRBD, TRBJ, TRAV, and TRAJ follow the IMGT gene nomenclature. The frequency is given as the number of clones with identical TCR rearrangements/total number of TCR clones sequenced.

cytotoxicity could be blocked with an antibody against TCR VB-11 but not with an isotype control antibody (Fig 5, D).

T-cell clones expressing drug-specific TCRs showed a CBZ- and HLA-B*1502-restricted response

To identify the functional combinations of VA/VB pairs of TCR, we generated 19 T-cell clones. Seven clones showing an upregulated expression of granulysin (>20 ng/mL) on CBZ

stimulation were selected for the TCR sequence analysis. The 7 clones have different combinations of CDR3 in the VA and VB chains, including 1 clone using VA-22/VB-15, 2 clones using VA-3/VB-11, and 4 clones using VA-22/VB-11 (Fig 6, A). Five clones with a different combination of VA/VB were selected for examining the cytotoxic response, and 2 clones (CS-10 and CS-11) possessing the combination of VB-11-ISGSY and VA-22 showed the most significant response against the target cells expressing the HLA-B*1502 molecule (Fig 6, B). In particular,

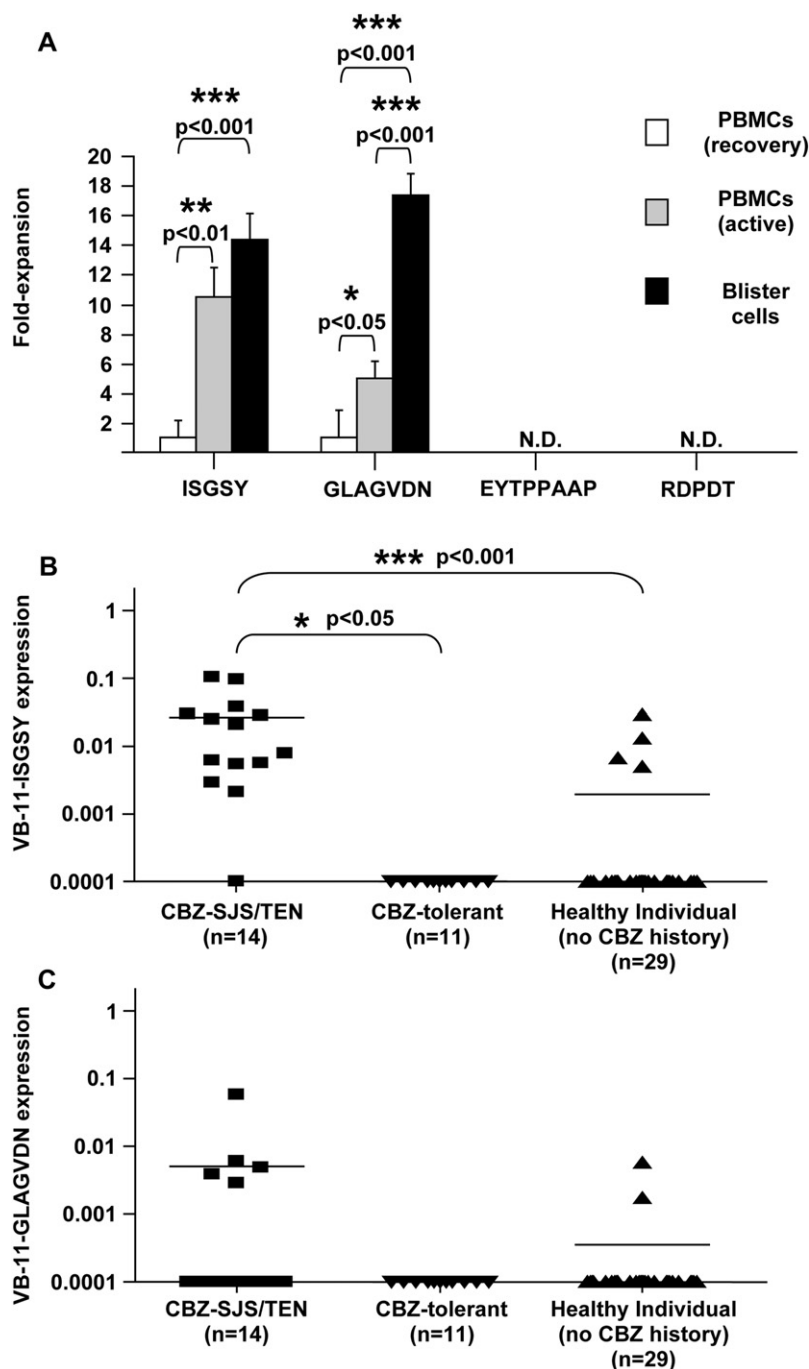


FIG 4. Correlation of CBZ-specific CDR3 clonotypes in patients with CBZ-SJS/TEN and disease onset. **A**, Expression levels of 2 TCR clonotypes (VB-11-ISGSY and VB-11-GLAGVDN) in PBMCs or blister fluid cells of patients ($n = 3$) in the indicated disease stage of CBZ-SJS/TEN was determined by using real-time PCR. **B** and **C**, Levels of CBZ-specific TCR clonotypes increased in PBMCs from patients with CBZ-SJS/TEN ($n = 14$) yet were absent in CBZ-tolerant control subjects ($n = 11$) and showed different degrees of increase in healthy subjects ($n = 29$). Each point of data (Fig 4, B and C) represents the expression levels of the clonotype of 1 subject. N.D., Not detectable. * $P < .05$, ** $P < .01$, and *** $P < .001$, unpaired t test.

clone CS-10, expressing a TCR pair comprised of VA-22-FISGTY and VB-11-ISGSY, showed the strongest cytotoxic response (Fig 6, B).

To determine whether the public TCR sequences confer specific receptors to recognize the HLA-B*1502/peptide/drug complex, we further established TCR transfectants by means of cotransfection of

cDNA fragments encoding the α - and β -chains of TCR and CD8 α/β chains in the Jurkat T-cell line (Fig 7 and see Figs E3, E4, and E5 in this article's Online Repository at www.jacionline.org). More than 98% of the cells expressed the transfected TCR, as examined by using the indicated mAb (see Fig E3). Coculturing with TCR transfectants (TF-10) and antigen-presenting cells expressing the

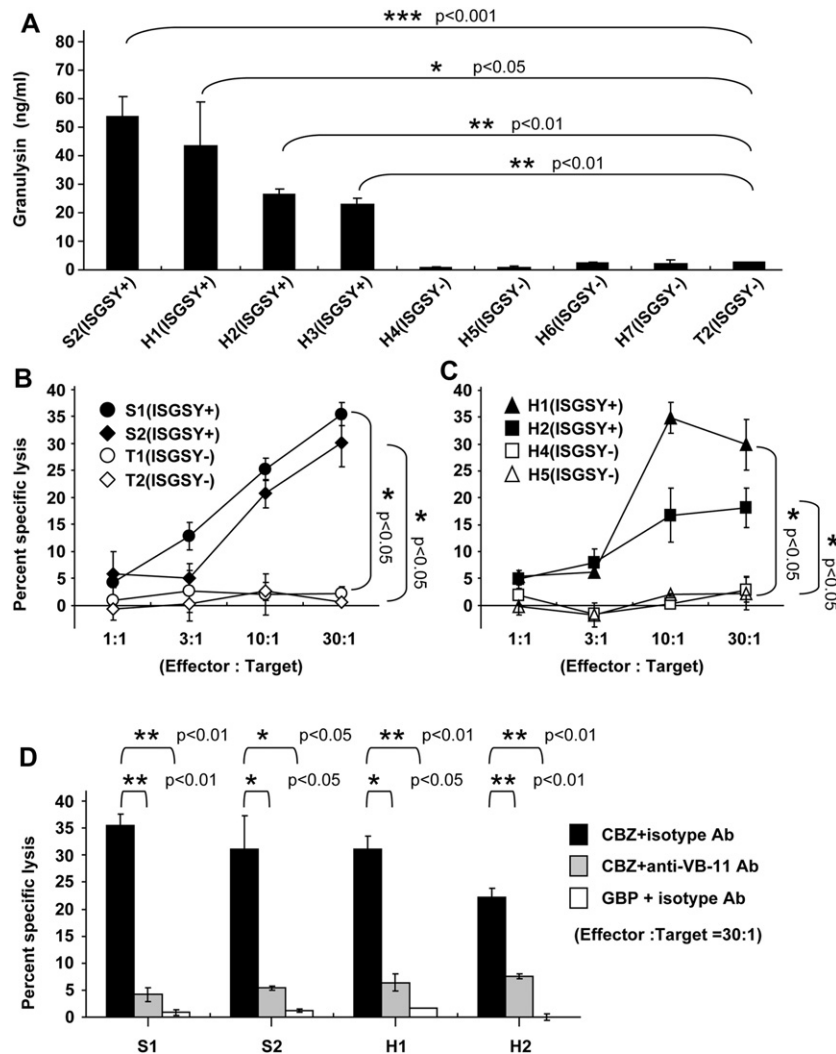


FIG 5. *In vitro* priming of disease-specific clonotypes and blockade of CBZ-specific cytotoxicity with anti-TCR antibodies. **A**, Increased release of granulylin by the CBZ-priming T cells (after 2 cycles of culture with CBZ) from a patient with CBZ-SJS (S2) and 3 healthy donors (H1-H3) but absent in 4 healthy donors (H4-H7) and a tolerant control subject (T2) who did not have VB-11-IGSY clonotypes. **B** and **C**, Cytotoxicities of the CBZ-priming T cells isolated from different subjects after 2 cycles of culture with CBZ were determined by using ^{51}Cr release assays. **D**, The CBZ-priming T cells were incubated with anti-TCR-VB-11 mAb or isotype control antibodies before the cytotoxicity assay. Ab, Antibody. * $P < .05$, ** $P < .01$, and *** $P < .001$; data in Fig 5, A and D, by unpaired *t* test and data in Fig 5, B and C, by paired *t* test.

HLA-B*1502 molecule, significant IL-2 secretion was observed in the supernatants on treatment with CBZ but not with GBP (see Fig E4). The transfectants (TF-10) exhibited the strongest response against the target cells expressing HLA-B*1502 rather than the parental C1R cells or cells expressing other HLA-B alleles, such as HLA-B*5801 (see Fig E4).

Cross-reactivity of TCR transfectants to CBZ and OXC

We expressed the CBZ-specific TCR clonotypes and CD8 in Jurkat T cells (TF-2, TF-4, TF-8, TF-10, and TF-11) to study the cross-reactivity between CBZ and OXC. The TCR transfectants responded to not only CBZ but also OXC, although to a lesser extent (Fig 7 and see Fig E5). As shown in Fig 7, an incubation of CBZ with a concentration of 100 $\mu\text{mol/L}$ induced TF-10 TCR

transfectants to produce a maximal IL-2 level (105 pg/mL), and OXC with 800 $\mu\text{mol/L}$ stimulated maximal IL-2 production (89 pg/mL), respectively. The half-maximal response of CBZ and OXC in TF-10 TCR transfectants were about 50 and 400 $\mu\text{mol/L}$, respectively (see the Methods section in this article's Online Repository). The reactivity to OXC and CBZ could also be found in 4 other TCR transfectants (TF-2, TF-4, TF-8, and TF-11; see Fig E5) but not observed in the control TCR Jurkat cells. TCR transfectants (TF-10 and TF-11) expressing the public sequences (VB-11-IGSY and VA-22) induced a stronger IL-2 release than other transfectants (TF-2, TF-4, and TF-8).

DISCUSSION

In this study we showed that CBZ-specific T cells (primarily CD8^+) could be expanded *in vitro* and exhibited cytotoxicity. We

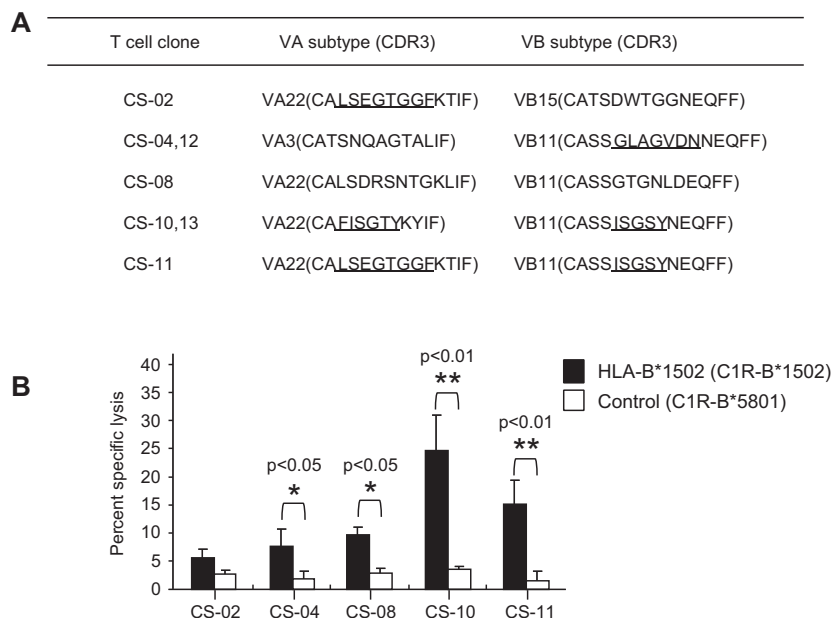


FIG 6. T-cell clones validate the functional role of TCRs in patients with CBZ-SJS/TEN. **A**, Amino acid sequences of VA and VB CDR3 clonotypes of 7 T-cell clones (CS-02, 04, 08, and 10-13) from patients with CBZ-SJS/TEN. **B**, Cytotoxicity was determined by incubating T-cell clones with CBZ (25 μ g/mL) and ^{51} Cr-loaded C1R-B*1502 or C1R-B*5801 cells. * $P < .05$ and ** $P < .01$, unpaired t test. See the [Methods](#) section in this article's Online Repository.

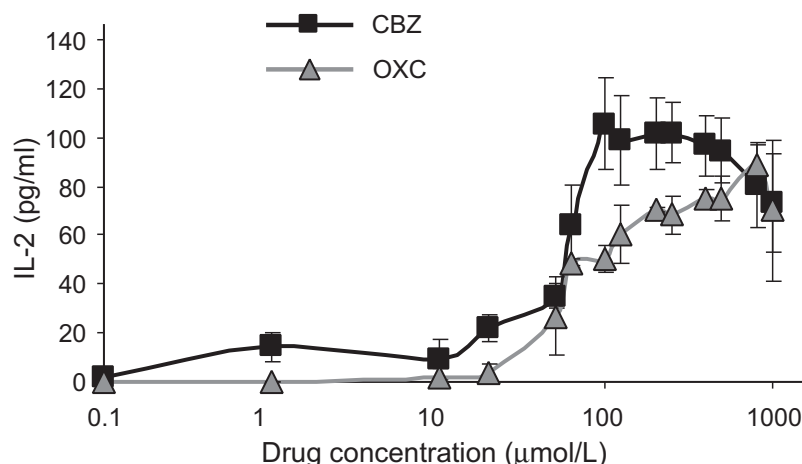


FIG 7. Patterns of cross-reactivity with CBZ and OXC in the TCR transfectants expressing disease-associated clonotypes. We determined the levels of IL-2 secretion in the culture supernatants of the TF-10 TCR (from CS-10 single clone) transfectants obtained from a 24-hour incubation with indicated concentrations (in micromoles) of CBZ or OXC by means of ELISA. The assay sensitivity for IL-2 was 4 pg/mL. Data are expressed as means $\pm$ SDs of triplicate measurements per condition and representative of 3 independent experiments.

demonstrated the restricted TCR repertoire use of these drug-specific CTLs and identified the predominant clonotypes shared by the patients. More importantly, the common clonotypes could also be detected in blister fluid cells or unstimulated PBMCs obtained directly from patients with CBZ-SJS/TEN or healthy subjects carrying the HLA-B*1502 allele. These HLA-B*1502 carriers contained unique subsets of oligoclonal T cells (eg, VB-11-ISGSY or VB-11-GLAGVDN), which were already present in the body before drug exposure and elicited robust immune reactions on CBZ antigenic stimulation. These data correlated

with the clinical observation and might explain why some HLA-B*1502 carriers are tolerant to CBZ. In addition, the TCR transfectants and anti-TCR antibody blocking further validated the pathogenic role of immunodominant TCRs in patients with CBZ-SJS/TEN. These data suggested that CBZ-specific HLA-B*1502-restricted CTLs shared common TCR clonotypes, recognized CBZ/peptide-HLA-B*1502 complex, and triggered life-threatening drug hypersensitivity reactions: SJS and TEN.

The importance for a specific TCR receptor clonotype has been studied in the pathophysiology of several HLA-associated

autoimmune disorders.³⁰ In this study we showed that in addition to the specific HLA-B*1502 allele, the presence of a T-cell repertoire containing the public sequences (VB-11-ISGSY, VB-11-GLAGVDN) could be another key factor for development of severe drug hypersensitivity. We detected a major public VB-11-ISGSY clonotype from PBMCs of patients with CBZ-SJS/TEN, as well as healthy subjects (with no history of CBZ administration) with CBZ-specific T cells (Fig 4, A and B). Our TCR transfectants confer the drug specificity of receptors to recognize the HLA-B*1502/peptide/drug complex, and the different responses of TCR transfectants to CBZ and OXC could be explained by distinct characteristic of each TCR. The association between the CBZ unreactivity and absence of public clonotypes of the T cells from drug-tolerant subjects or healthy subjects could be explained by the lack of CBZ-specific T cells with sufficient avidity required to generate granulysin secretion and CTL activity. Therefore our data suggested that the correct combination of TCR, drug, peptide, and HLA is crucial for driving drug hypersensitivities.

Formation of the immune synapse by HLA-drug-TCR is the key for the initiation of the immune response, as well as drug hypersensitivity. However, how TCR diversity was managed in patients with drug hypersensitivity was not well understood. The preferred TCR clonotypes identified in this study might represent the molecular fingerprint of T cells that recognize the small drug/peptide-HLA-B*1502 complex.^{4,8} A limited yet distinct TCR use might depend on the TCR avidity for CBZ/peptide-HLA-B*1502 complex among patients with CBZ-SJS/TEN. However, the drug-specific clonotypes were not present in all of the patients. For example, 2 of 19 patients with SJS/TEN without the clonotypes (VB-11-ISGSY and VB-11-GLAGVDN) had the disease. Further studies are required to examine the role of whole drug-specific TCR clonotypes in the pathogenesis of hypersensitivity reactions.

Why are there CBZ-specific TCR clonotypes in some subjects but not others who carry HLA-B*1502? This might be explained by random TCR recombination, the different T cells' clonal expansion/deletion caused by the subjects' distinct immunologic histories, antigen loading and the microenvironment in the thymus, clonal suppression, anergy and deletion of T cells, interactions between regulatory and conventional T cells, individual differences in peripheral tolerance, polymorphisms of genes that are involved in drug metabolism, immune reactions, gene rearrangements for generating TCR repertoires, and TCR revisions.^{31,32} In addition, other risk factors might be involved in drug hypersensitivity. For example, subjects with a prior medical history of viral infection (eg, HIV and human herpesvirus) or autoimmune disorders (eg, systemic lupus erythematosus) have been noted to be more susceptible to drug hypersensitivity.^{33,34}

Because OXC is the prodrug of CBZ and they share structural similarity, the cross-reactivity between CBZ and OXC to T cells has been examined in patients, and it has been reported that 33% of the patients who had a CBZ-induced rash also had OXC hypersensitivity.³⁵ T cells isolated from patients with CBZ hypersensitivity showed cross-reactivity to OXC *in vitro*.²⁴ In this study we found that CBZ-specific TCR clonotypes showed cross-reactivity to OXC, although to a lesser extent (Fig 7 and see Fig E5). In addition, the T cells from OXC-tolerant or CBZ-tolerant control subjects showed similar characteristics with respect to the insufficient reactivity to CBZ despite incubation with antigen-presenting cells expressing the HLA-B*1502 molecule (Fig 3 and see Fig E1). However, considering CBZ and OXC

have different metabolism pathways in human bodies,³⁶ we cannot exclude the possibility that the metabolites of CBZ and OXC might possess different potential of antigenicity and distinct affinity for T-cell receptors, leading to the activation of T cells with different repertoires. This hypothesis could be supported by the observation that the incidences of hypersensitivity reactions caused by these 2 drugs were different, and SJS/TEN cases caused by OXC were reported to be fewer than those caused by CBZ.³⁷⁻³⁹ Global analysis of TCR repertoires in patients with OXC-induced hypersensitivity in the future will provide a piece of important information to clarify the contribution of T cells in cross-reactivity between CBZ and OXC.

In summary, this study established the key role of the TCR in the pathogenic mechanisms of SJS/TEN, revealed the correlation between the presence of TCR clonotypes and the clinical status of drug hypersensitivity, and provided a biomarker profile for CBZ-SJS/TEN.

Clinical implications: The drug-specific TCRs of CTLs play a key role in the pathogenic mechanism of SJS/TEN, which provides a biomarker profile for the diagnosis of drug hypersensitivity.

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METHODS

Chemicals and generation of T-cell clones

PBMCs were isolated from heparinized blood by means of Ficoll-Isopaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient separation. A stock CBZ (molecular weight, 236 daltons) solution and OXC (molecular weight, 252 daltons) were prepared in dimethyl sulfoxide and diluted with culture medium before use. The CD8⁺ T cells were sorted by using CD8 mAb (RPA-T8, BD Biosciences) and cloned by means of limiting dilution after the incubation of IL-2 (100 U/mL) in the culture medium and stimulation with irradiated autologous (or allogeneic) PBMCs or B-LCLs expressing the HLA-B*1502 molecule every 14 days.

Determination of drug-specific cytotoxicity *in vitro*

Incubation of target cells with medium alone or with 5% Triton X-100 was used to determine spontaneous and maximal release, respectively. GBP (25 µg/mL), an anticonvulsant with no structural similarity to CBZ, was used as the control. For the blocking experiments, T cells were incubated with an anti-TCR-VB-11 mAb (C21, Immunotech) or IgG_{2a} control (X39, BD Biosciences) of 6.25 µg/mL in the culture medium at 4°C for 30 minutes before standard cytotoxicity assays.

TCR repertoire analysis

cDNA fragment length was analyzed with an ABI 3730 Analyzer and GeneMapper Analysis Software 3.0 (Applied Biosystems, Foster City, Calif). We amplified the PCR products of TCR genes by using sequence-specific PCR primers, cloned them into a pGEM-T easy vector (Promega, Madison, Wis), and then picked at least 30 colonies from each patient for sequencing analysis by using an ABI PRISM 3730 DNA Analyzer and the ImMunoGeneTics information system (www.imgt.org), as previously described.^{E1}

Quantitative real-time PCR

The sets of oligonucleotide primers for quantifying the expansion fold of VB, VA, and particular clonotypes are listed in [Tables E4 and E5](#). The plasmid

template for the real-time PCR standards was the same used in CDR3 sequencing. Each primer/probe set was tested against the target plasmids and redesigned, if necessary, to prevent cross-reactivity. The expression level of the specific TCR clonotype was normalized by that of CD3, and the detection limitation of the ratio of TCR clonotype/CD3 was 0.0001. Real-time PCR with cDNA samples used a Light Cycler Fast Start DNA Master SYBR Green I Kit (Roche, Mannheim, Germany), according to the manufacturer's instructions. The number of cycles necessary to reach threshold fluorescence for each gene or β-actin control primer reaction was calculated as the crossing point (cycle threshold), and the cycle threshold of CD3 or β-actin in each reaction was used as the loading control in parallel experiments.

Antigen titration and IL-2 secretion assay

We expressed the 5 CBZ-specific TCR clonotypes identified from the single clones (CS-2, CS-4, CS-8, CS-10, and CS-11) and CD8 in Jurkat T cells. A wide titration of antigens (CBZ or OXC) was performed with the 5 TCR transfectants (TF-2, TF-4, TF-8, TF-10, and TF-11) and CBZ (or OXC)-pulsed/HLA-B*1502-transfected C1R cells. The reactivity of these TCR transfectants to CBZ and OXC was compared by using drug concentrations ranging from 0.1 to 1000 µmol/L. The half-maximal response was calculated based on IL-2 production. We incubated TCR transfectants (5.0×10^4), target cells (5.0×10^4 , irradiated 50 Gy), and indicated antigen concentrations (range, 0.1-1000 µmol/L) in the complete RPMI medium (200 µL) for 24 hours. The amount of human IL-2 released in the medium was measured with a human IL-2 ELISA kit (eBioscience, San Diego, Calif). The reactivity of the TCR transfectants to CBZ and OXC was evaluated by determining the concentrations of drug needed to obtain the half-maximal response values from the concentration-response curves.

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CDR3 spectratyping analysis of VB in drug-tolerant individuals

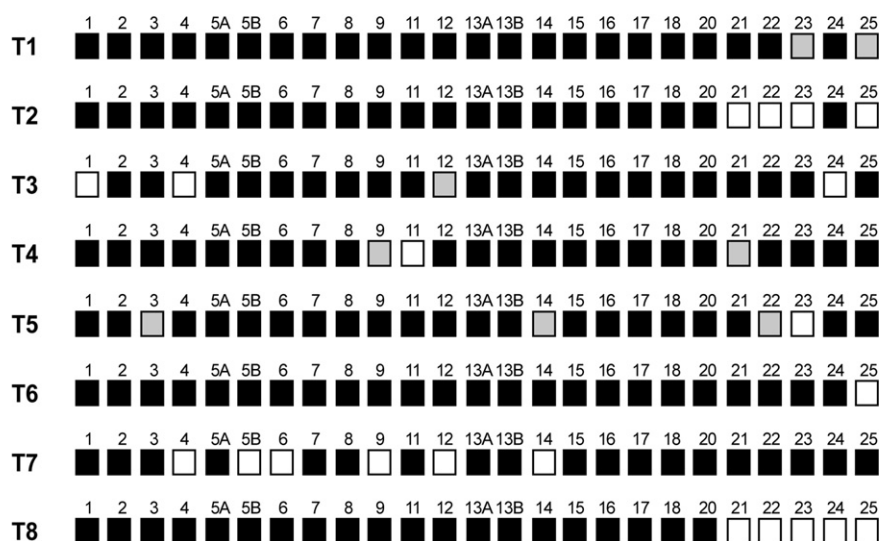


FIG E1. TCR VB repertoires in CBZ-stimulated T cells obtained from drug-tolerant subjects. CDR3 spectratype patterns of CD8⁺ cells were obtained from CBZ-stimulated T cells of CBZ/OXC-tolerant subjects ($n = 8$) at the second cycle of CBZ incubation. *Black squares* indicate a normal CDR3 size profile (quasi-Gaussian distribution, >5 peaks), *gray squares* indicate a restricted CDR3 size profile (skewed TCR repertoire, 1-4 peaks), and *open squares* indicate an undetectable CDR3 size profile (undetectable TCR, 0 peak). Data were obtained by using duplicate measurements of the PCR products with the cDNA of T-cell expansions of 8 donors and are representative of 2 independent experiments.

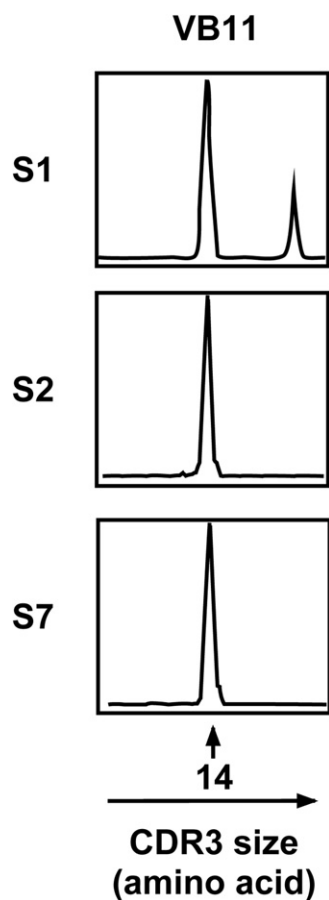


FIG E2. Spectratype profiles of skewed TCR VB-11 with identical CDR3 length. CDR3 spectratype patterns of the VB-11 subfamily of CBZ-stimulated CD8⁺ T cells were obtained from the fifth cycle of *in vitro* cultures from patients with CBZ-SJS (S1, S2, and S7). The *x-axis* displays CDR3 length (amino acid), and the *y-axis* indicates the fluorescence intensity of the runoff products of VB-11. Data are representative of at least 2 independent experiments that measured the PCR products of T-cell expansions of 3 donors.

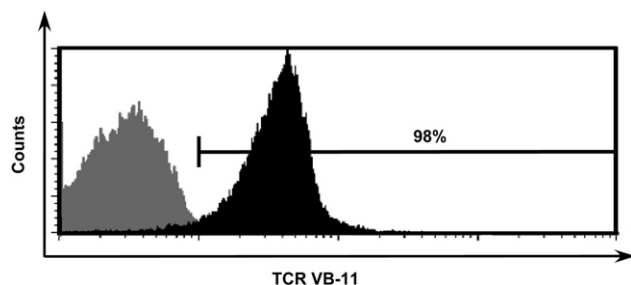


FIG E3. Expression of CBZ-specific T-cell receptor in Jurkat T cells. The full-length cDNA fragments encoding TCR VA-22 and VB-11 of the T-cell clone (CS-10) were amplified by means of PCR with specific primers (Tables E4 and E5). The expression vectors containing TCR VA-22, VB-11, and CD8 cDNA fragments, respectively, were cotransfected into the Jurkat T-cell line. The transfected Jurkat cells were maintained in the culture medium containing 10 μ g/mL blasticidin. The TCR transfectant (TF-10, VA-22-FISGTY, and VB-11-ISGSY; *black histogram*) and control parental cells (*gray histogram*) were stained with anti-VB-11 mAb and quantified by means of flow cytometry. The *number above the bracketed line* indicates the percentage of TCR VB-11-expressing cells. Data are representative of at least 2 independent experiments.

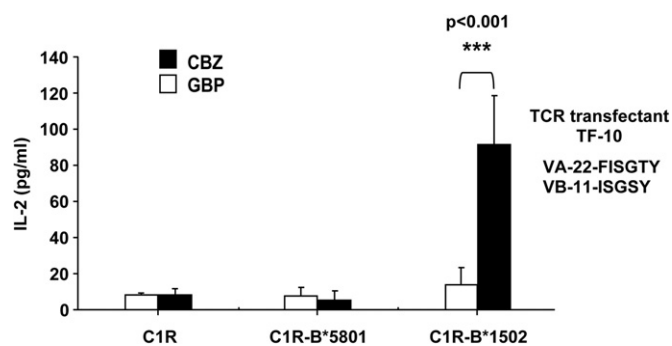


FIG E4. IL-2 secretion of TCR transfectants on stimulation with CBZ. The TF-10 (VA-22-FISGTY and VB-11-ISGSY) TCR transfectants with CD8 expression were incubated with parental C1R cells or C1R cells expressing HLA-B*1502 (C1R-B*1502 cells) or HLA-B*5801 (C1R-B*5801 cells) in the presence of CBZ (50 μ g/mL) or GBP (50 μ g/mL). We determined the levels of IL-2 secretion in Jurkat cell-culture supernatants obtained from a 24-hour incubation with drug stimulation (CBZ of 50 μ g/mL or GBP of 50 μ g/mL) by using an ELISA kit. The assay sensitivity for IL-2 was 4 pg/mL. Data are expressed as means $\pm$ SDs of triplicate measurements per condition and are representative of at least 2 independent experiments. *** P < .001, unpaired t test.

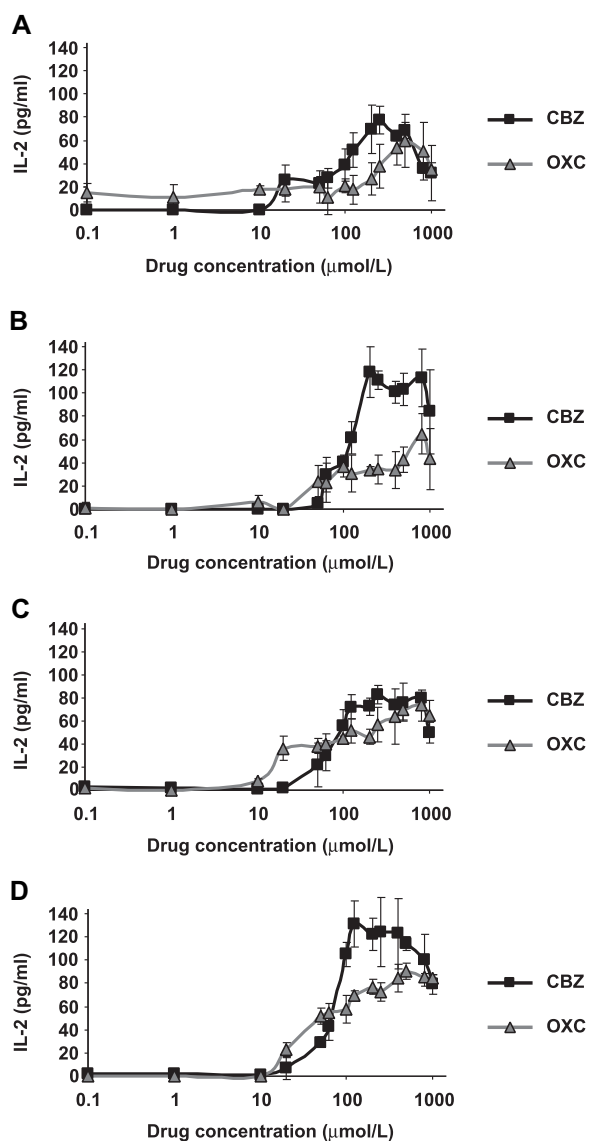


FIG E5. The reactivity of TCR transfectants to CBZ and OXC. We determined levels of IL-2 secretion in the culture supernatants of the TCR transfectants TF-2 (A), TF-4 (B), TF-8 (C), and TF-11 (D) after a 24-hour incubation with the indicated concentration (in micromoles) of CBZ or OXC. The assay sensitivity of ELISA for IL-2 was 4 pg/mL. Data are expressed as means $\pm$ SDs of triplicate measurements per condition and are representative of at least 2 independent experiments.

TABLE E1. The HLA-B genotype and clinical information of study subjects

Case no.	HLA-B*	Type of drug hypersensitivity	Disease stage	Drug
S1	1502/3802	SJS	Recovery	CBZ
S2	1502/4601	SJS	Recovery	CBZ
S3	1502/8101	SJS	Recovery	CBZ
S4	1502/5401	Overlapping SJS and TEN	Recovery	CBZ
S5	1502/1302	SJS	Active	CBZ
S6	1502/5801	SJS	Active	CBZ
S7	1502/1301	SJS	Active	CBZ
S8	1502/1302	SJS	Active	CBZ
S9	1502/1301	SJS	Recovery	CBZ
S10	1502/1501	SJS	Recovery	CBZ
S11	1502/1801	SJS	Recovery	CBZ
S12	1502/4001	SJS	Recovery	CBZ
S13	1502/5101	SJS	Recovery	CBZ
S14	1502/4001	SJS	Recovery	CBZ
S15	1502/6701	SJS	Recovery	OXC
S16	1502/4601	SJS	Recovery	CBZ
S17	1502/1301	SJS	Recovery	CBZ
S18	1502/4001	SJS	Recovery	CBZ
S19	1502/0702	SJS	Recovery	CBZ
T1	1502/4001	Tolerant	Not applicable	CBZ
T2	1502/4601	Tolerant	Not applicable	CBZ
T3	1502/5801	Tolerant	Not applicable	OXC
T4	1502/1501	Tolerant	Not applicable	OXC
T5	1502/3501	Tolerant	Not applicable	OXC
T6	1502/5502	Tolerant	Not applicable	OXC
T7	1502/4001	Tolerant	Not applicable	OXC
T8	1502/4601	Tolerant	Not applicable	OXC
T9	Absence of B*1502	Tolerant	Not applicable	CBZ
T10	Absence of B*1502	Tolerant	Not applicable	CBZ
T11	Absence of B*1502	Tolerant	Not applicable	CBZ
T12	Absence of B*1502	Tolerant	Not applicable	CBZ
T13	Absence of B*1502	Tolerant	Not applicable	CBZ
T14	Absence of B*1502	Tolerant	Not applicable	CBZ
T15	Absence of B*1502	Tolerant	Not applicable	CBZ
T16	Absence of B*1502	Tolerant	Not applicable	CBZ
T17	Absence of B*1502	Tolerant	Not applicable	CBZ

*HLA-B alleles were determined by using sequence-specific oligonucleotide reverse line blots (DYNAL Biotech Ltd, Bromborough, United Kingdom). Potential ambiguities were resolved by means of sequencing-based typing, as described previously.^{E2} The sequence data were analyzed with SeqScape v2 (Applied Biosystems).

TABLE E2. Primers for TCR VB CDR3 spectratyping analysis

Primers	Sequences (5' to 3')
VB1	CGCACAAACAGTTCCCTGACT
VB2	TCAACCATGCAAGCCTGA
VB3	GTCTCTAGAGAGAAGAAGGAGCGC
VB4	GAGGCCACATATGAGAGTGG
VB5A	TCAGTGAGACACAGAGAAAC
VB5B	TGTGTCCTGGTACCAACAGG
VB6	CTCAGGTGTGATCCCAATTTC
VB7	GCTCTCACCTGAATGCCCC
VB8	TCTGGTACAGACAGACCATG
VB9	CCTAAATCTCCAGACAAAGC
VB11	TCAACAGTCTCCAGAATAGGACG
VB12	CATGGGCTGAGGCTGATC
VB13A	CGACAAGACCCAGGCATGGG
VB13B	AGACAAGATCTAGGACTGGG
VB14	GTCTCTCGAAAAGAGAAGAG
VB15	GTGTCTCTCGACAGGCACAG
VB16	AGTCTAAACAGGATGAGTCCG
VB17	GGAGATATAGCTGAAGGGTA
VB18	GAGTCAGGAATGCCAAAGGA
VB20	CAGCTCTGAGGTGCCCCAGA
VB21	TCACAGTTGCCTAAGGATCG
VB22	GCAGAAAGTCGAGTTTCTGG
VB23	GCAGGGTCCAGGTCAGGACCCCCA
VB24	ACAATGAAGCAGACACCCCT
VB25	TAAGTGCCTCCCAAATTCAC
CB	GACAGCGGAAGTGGTTGCGGGGT
CB-FAM	FAM-TTGGGTGTGGGAGATCTCTGC

TABLE E3. Primers for TCR VA CDR3 spectratyping analysis

Primers	Sequences (5' to 3')
VA1A	TCTGGTATGTGCAATACCCCAACC
VA1B	CTGAGGAAACCCTCTGTGCA
VA2	GATGGAAGGTTTACAGCACAGCTC
VA3	CACAGTGGAAGATTAAGAGTCACGC
VA4A	AACAGAATGGCCTCTCTGGC
VA4B	GGATTGCGCTGAAGGAAGAG
VA5	TGAAGGTCACCTTGATACCACCC
VA6	AATCCGCCAACCTTGTCATCTCCG
VA7	AACTGCACGTACCAGACATC
VA8	ACCCTGAGTGTCCAGGAGGG
VA9	CACTGCTGACCTTAACAAAGGCG
VA10	TCCTGGTGACAGTAGTTACG
VA11	AGGCTCAAAGCCTTCTCAGCAGGG
VA12	TCCACCAGTTCCTTCAACTTCACC
VA13	TTCATCAAAACCCTTGGGGACAGC
VA14	CCCAGCAGGCAGATGATTCTCGTT
VA15	GGATAAACATCTGTCTCTGCG
VA16	GATAGCCATACGTCCAGATG
VA18	TGCCACTCTTAATACCAAGGAGGG
VA19	ACACTGGCTGCAACAGCATC
VA20	TTACAAACGAAGTGGCCTCC
VA21	ACCCTGCTGAAGGTCTTACATTCC
VA22	CTTGGAGAAAGGCTCAGTTC
VA23	TGCCTCGCTGGATAAATCATCAGG
VA24	TCCCAGCTCAGCGATTCTCAGCTCC
VA25	GTCTGTCTCTTGATAGCC
VA26	AGCCCAGCCATGCAGGCATCTACC
VA27	TTGATACCAAAGCCCGTCTC
VA28	GAACATCACAGCCACCCAGACCGG
VA29	GCAAAGCTCCCTGTACCTTACGG
VA30	TTTCTGCACAGCACAGCCCC
VA31	AGCAAAAACCTTCGGAGGCGG
VA32	AAGGAGAGGACTTCACCACG
CA	GTTGCTCTTGAAGTCCATAGACC
CA-FAM	FAM-GCAGACAGACTTGTCCTGG

TABLE E4. Primers for TCR VB quantitative PCR analysis and cloning

Primers	Sequences (5' to 3')
Q-VB1	CACTCTGAACTAAACCTGA
Q-VB2	TCAACCATGCAAGCCTGACC
Q-VB3	CGCTTCTCCCTGATTCTGGAGTCC
Q-VB4	TTCCCATCAGCCGCCCAAACCTA
Q-VB5A	CTGAGATGAATGTGAGCACCTTG
Q-VB5B	CTGAGCTGAATGTGAACGCCTTG
Q-VB6	AGATCCAGCGCACAGAGCG
Q-VB7	GCCAAGTCGCTTCTCACCTG
Q-VB8	TGAAGATCCAGCCCTCAGAACCC
Q-VB9	TCTACCTAAATCTCCAGACAAAG
Q-VB11	TGCCAGGCCCTCACATACCTCTCA
Q-VB12	GAGAATTTCTCCTCACTCTGG
Q-VB13A	CTCAGGCTGCTGTCGGCTG
Q-VB13B	CTCAGGCTGGAGTTGGCTG
Q-VB14	AGGGTACAAAGTCTCTCGAAAAG
Q-VB15	CAGGCACAGGCTAAATCTCTCC
Q-VB16	GAACTGGAGGATTCTGGAGT
Q-VB17	GAAGGGTACAGCGTCTCTCGG
Q-VB18	TTTCTGCTGAATTTCCCAAAGAGG
Q-VB20	AGGTGCCCCAGAATCTCTCAG
Q-VB21	TCAAAGGAGTAGACTCCACTCTC
Q-VB22	AGATCCGGTCCACAAAGCTG
Q-VB23	ATTCTGAACTGAACATGAGCTCCT
Q-VB24	ATCCAGGAGGCCGAACACTTC
Q-VB25	TAAGTGCCTCCCAAATTCAC
Q-CB	GGTGTGGGAGATCTCTGCTTC
Q-VB-11-ISGSY	TTGTAGGAACCTGAGATGGAA
Q-VB-11-GLAGVDN	TTGTGTCAACTCCCGCTAACCCACT
VB-11-Fw	ATTAGGTACCATGACTATCAGGCTCCTCTG
VB-Re	CTAGCCTCTGGAATCCTTT

TABLE E5. Primers for TCR VA quantitative PCR analysis and cloning

Primers	Sequences (5' to 3')
Q-VA1A	GGTATGTGCAATACCCCAACC
Q-VA1B	CTGAGGAAACCCTCTGTGCA
Q-VA3	ACAGTGGAAGATTAAGAGTCACGC
Q-VA4A	AACAGAATGGCCTCTCTGGC
Q-VA12	CACCAGTTCCTTCAACTTCACC
Q-VA14	CAGCAGGCAGATGATTCTCGT
Q-VA22	GGAGAAAGGCTCAGTTCAAG
Q-VA23	CCTCGCTGGATAAATCATCAG
Q-VA26	CCAGCCATGCAGGCATCTAC
Q-CA	TCAGGGTTCTGGATATTGGGTA
VA-22-Fw	ACACTAAAGATGAACTATTCTCCAGGCT
VA-Re	TCAGCTGGACCACAGCCGCAGCGT