Structural modeling of HLA-B*1502/peptide/carbamazepine/T-cell receptor complex architecture: implication for the molecular mechanism of carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis

Peng Zhou*, Shilei Zhang, Yewang Wang, Chao Yang and Jian Huang

Center of Bioinformatics (COBI), Key Laboratory for Neuroinformation of the Ministry of Education, Center for Information in BioMedicine, School of Life Science and Technology, University of Electronic Science and Technology of China (UESTC), Chengdu 610054, China

Communicated by Ramaswamy H. Sarma

(Received 20 May 2015; accepted 7 September 2015)

1. Introduction

Adverse drug reactions (ADRs) cause significant morbidity and mortality for patients and are an expense to the healthcare system. It was reported that more than two million hospitalized patients/year who experience a serious ADR and 106,000/year who die from an ADR in the United States (Bond & Raehl, 2006); the relevant cost has been estimated at US$136 billion annually (Johnson & Bootman, 1995). The ADRs can be classified into types A and B. Type A reactions are associated with drug’s pharmacological activity; they are dose-dependent and are therefore readily reversible on reducing the dose or withdrawing the drug. In contrast, type B adverse reactions are caused by immune response and cannot be predicted from the known pharmacology of drugs (Pirmohamed, Brekenridge, Kitteringham, & Park, 1998).

In the past years, intensive efforts addressed to seek predictive markers of type B ADRs have led to the discovery of numerous ADR associations with diverse alleles of the human leukocyte antigen (HLA) genes, which are functionally related to the T-cell activation and following cytotoxicity (Illing, Vivian, Purcell, Rossjohn, & McCluskey, 2013). Recently, hundreds of drug–HLA–ADR association pairs were collected via an exhaustive survey of publicly available sources, which contains FDA-approved drugs, unique HLA alleles and different categories of ADRs from published literatures. From the collection, the strongest associations have been identified as carbamazepine (CBZ)-induced Stevens–Johnson syndrome/toxic epidermal necrosis (SJS/TEN) in the presence of HLA-B*1502 (OR = 1357, 95% CI: 193 to 8838) (Chen et al., 2011), allopurinol-induced SJS/TEN/hypersensitivity syndrome (HSS) in presence of HLA-B*5801 (OR = 580, 95% CI: 34–9781) (Hung et al., 2005) and abacavir hypersensitivity (OR = 117, 95% CI: 29–481) (Mallal et al., 2002) or fluclaxacillin-induced

*Corresponding author. Email: p_zhou@uestc.edu.cn

© 2015 Informa UK Limited, trading as Taylor & Francis Group
In spite of strong genetic associations, the molecular mechanism of drug interactions with specific HLA alleles is not yet well characterized. Several theories, including hapten (or prohapten), p:i (immune receptor) and danger models, have been explained to illustrate the HLA-associated drug hypersensitivity reactions (HSR) (Bharadwaj et al., 2012; Pirmohamed, Naisbitt, Gordon, & Park, 2002). However, it is hard to determine whether these theories can be used for a specific reaction and, if yes, which one is applicable to the reaction. Recently, the high-resolution crystal structures of self-peptides presented by abacavir-modified HLA-B*5701 have been solved (Illing et al., 2012; Ostrov et al., 2012), which gave the first molecular-level insight into structural basis of HLA-associated drug hypersensitivity (Pompeu et al., 2012). The crystallographic study, however, is extremely time-consuming and expensive, thus limiting its application even for a very few reported cases. Instead, the structural bioinformatics approach, which has been rapidly progressed in recent years, provides a promising way to computationally elucidate the structural basis, dynamics behavior, and energetic property of HLA–drug association and their biological role in diverse ADRs. Nevertheless, although the strategy has been widely applied to investigating various biological phenomena, it, to the best of our knowledge, still remains unexploited in the ADR area.

In this study, we attempt to understand the molecular mechanism and pathological implication underlying the HLA-B*1502-associated, CBZ-induced SJS/TEN. The CBZ is an anticonvulsant and mood-stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder as well as trigeminal neuralgia, which has also been observed to cause several serious adverse reactions, including maculopapular exanthema, HSS and the SJS/TEN (Chung & Hung, 2012). The CBZ-induced SJS/TEN is estimated to be responsible for up to 35% of drug-induced SJS/TEN cases in the Han Chinese population, but only 6% of cases in Caucasians, which occurs within 2 months of drug administration, with a median onset of 15 days (Bharadwaj, Illing, & Kostenko, 2010). The nearly 100% association of HLA-B*1502 with CBZ-induced SJS/TEN suggests that the HLA-B*1502 is a central participant in the pathogenesis of the disease (Yang et al., 2007). Here, we attempt to computationally model the atomic-level structure of the complete HLA-B*1502/peptide/CBZ/T-cell receptor (TCR) complex by integrating theoretical methods and empirical knowledge. We demonstrated that the modeled complex can be readily utilized to explain most observations in previous reports. In addition, based on the model, two hypotheses were also proposed that may help to guide next wet-lab experiments.

2. Materials and methods

2.1. Virtual mutation of amino acid residues

The few focused amino acid residues in HLA, peptide, or TCR can be virtually mutated to other residue types using an integrative protocol: first, the side chains of the focused residues were removed manually from structure architecture and then new side chains were added automatically to these incomplete residues using the rotamer-based SCWRL program (Krivov, Shapovalov, & Dunbrack, 2009). The SCWRL algorithm predicted all possible combinations of side chain rotamers in a specific structural context using tree decomposition strategy and optimized topological parameters for the interaction graph between these combinations based on a backbone-dependent rotamer library derived from high-quality protein crystal structures. Previous comparison studies demonstrated that the SCWRL performed much better than other available tools such as SCATD, SPDBV, and SCit in predicting the side chain conformations of HLA/peptide complexes (Knapp et al., 2008). In addition, the SCWRL has recently been successfully utilized to reproduce the crystal structures of peptide side chains in receptor-bound state (Zhou, Wang, Ren, Yang, & Tian, 2013; Zhou, Yang, Ren, Wang, & Tian, 2013). We therefore believe that this rotamer-based method can be applied to the current system as well.

2.2. Molecular docking

The molecular docking described in our recent work (Yang, Wang, Zhang, Huang, & Zhou, 2015) was carried out using Autodock Vina (Trott & Olson, 2010). The CBZ molecule was constructed and optimized with the MMFF94 force field (Halgren, 1996), and Gasteiger partial charges (Gasteiger & Marsili, 1996) were assigned for its atoms. The polar hydrogen atoms and Kollman charges (Besler, Merz, & Kollman, 1990) were added to HLA protein and bound peptide using the AutoDock Tools (Morris et al., 2009), which was also utilized to prepare the input pdbqt file for the CBZ ligand and to set the size and center of a grid box covering the CBZ-binding site on HLA-B*1502/peptide complex surface, with the dimensions of x, y, and z in 1.0 Å spacing. In the docking procedure, the Lamarckian genetic algorithm was employed to generate thousands of potential conformations for CBZ within the box region, which were finally clustered into few representatives.

2.3. MD simulation

Important biological functions like protein conformational changes (Balasco, Barone, & Vitagliano, 2015), new molecule design (Arfeen, Patel, Khan, & Bharatam, in press), and structural features (Fan, Zheng, Cui, Li, &
Zhang, 2015) can be uncovered by investigating them using molecular dynamics (MD) simulations. Here, MD simulations were performed on the constructed HLA/peptide, HLA/peptide/CBZ, and HLA/peptide/CBZ/TCR systems using the AMBER10 force field implemented in Amber11 package (Case et al., 2005). The complex systems were solvated in a periodic TIP3P water box extended 10 Å away from any solute atom. Counter-ions of Na⁺ were placed around the solute molecules based on Coulombic potential to keep the whole systems neutral. The antechamber tool (Wang, Wang, Kollman, & Case, 2006) was used to treat CBZ molecule. In the procedure, the AM1-BCC charge (Jakalian, Jack, & Bayly, 2002) was assigned to each CBZ atom; the topology and connectivity were generated for CBZ molecular structure, and the general AMBER force field (Wang, Wolf, Caldwell, Kollman, & Case, 2004) was employed to describe the force field parameters that were not found in the standard AMBER.

The systems were minimized using a combination of steepest descent algorithm and conjugate gradient algorithm; first, all hydrogen atoms were minimized, followed by all water molecules and counter-ions, and finally the minimization was addressed on the whole systems without any constraint. The maximum number of minimization steps was set to 5000. After the minimizations, the systems were heated to 300 K in 300 ps followed by constant temperature equilibration at 300 K for 500 ps. Subsequently, 100-ns MD production simulations were carried out in an isothermal isobaric ensemble with periodic boundary conditions. An integration step of 2 fs was used for the MD simulations and the particle mesh Ewald method (Darden, York, & Pedersen, 1993) was employed to calculate the long-range electrostatic interactions. A cut-off distance of 10 Å was used to calculate the short-range electrostatics and van der Waals interactions. In order to restrain all covalent bonds involving hydrogen atoms, the SHAKE method (Ryckaert, Ciccotti, & Berendsen, 1977) was used. Each simulation was coupled to a 300 K thermal bath at 1.0 atm through the Langevin algorithm (Wu & Brooks, 2003).

2.4. Interaction free energy analysis

The mm-pbsa program in Amber11 package (Case et al., 2005) was used to analyze the interaction free energies between different parts of a complex system. The analysis was performed over the thousands of snapshots evenly extracted from the last 50 ns of MD trajectory. The total free energy change ∆G_{tot} upon the binding of two components consists of direct nonbonded interaction ∆G_{nbd} and indirect solvent effect ∆G_{solv} (Zhou, Huang, & Tian, 2012), i.e. ∆G_{tot} = ∆G_{nbd} + ∆G_{solv}; the former was calculated using the molecular mechanics (MM) approach based on AMBER10 force field, and the latter was expressed as solvation free energy, which can be decomposed into electrostatic and nonpolar contributions. The electrostatic contribution to solvation free energy was calculated by finite difference solution of nonlinearized Poisson–Boltzmann (PB) equation using the delphi program (Rocchia, Alexov, & Honig, 2001). In the calculation, the solute and solvent were assigned with dielectric constants 2 and 78, respectively. The atomic radii and partial charges were taken from the parse parameter set (Sitkoff, Sharp, & Honig, 1994) and the AMBER10 force field, respectively; the nonpolar solvation contribution was derived from the surface area (SA) model $\beta + \gamma A$, where $A$ is solvent accessible surface area computed with a solvent-probe radius of 1.4 Å, and $\beta$ and $\gamma$ are coefficient terms that were defined to be .92 kcal/mol and .00542 kcal/mol/Å², respectively, as suggested by Kollman and co-workers (Kollman et al., 2000).

3. Results and discussion

3.1. Analysis of epidemiological investigations and experimental evidences

The CBZ-induced SJS/TEN has been previously reported to strongly associate with HLA-B*1502 allele in Han Chinese populations (Chung et al., 2004). The nearly 100% association implied that the HLA-B*1502 is not only a genetic marker but also a participant in the pathogenesis of the disease (Yang et al., 2007). Later, from gene fine mapping, Hung et al. narrowed the susceptibility locus for the CBZ-induced SJS/TEN to within 86 kb region where HLA-B is the only known gene, and confirmed a very strong association of the adverse reaction with HLA-B*1502 (OR = 1357, 95% CI: 193 to 8838) (Hung et al., 2006).

Based on the epidemiological investigations, Chen and co-workers have performed a series of experimental analyses to give molecular insights into the strong association (Yang et al., 2007; Ko et al., 2011), and they found that (i) the antigen peptides of HLA-B*1502 prefer use of serine residues at their nonanchoring positions P5, P6, P7, and P8, (ii) CBZ does not covalently modify these peptides, (iii) CBZ does not alter the peptide repertoire of HLA-B*1502, (iv) HLA-B*1502 directly presents CBZ without intracellular metabolism and antigen processing, (v) the presentation of CBZ requires endogenous peptides loaded in the antigen-binding groove of HLA-B*1502, (vi) the cytotoxicity elicited by CBZ or its metabolites can be abolished by a washing procedure, (vii) the 5-carboxamide moiety is functionally required for CBZ and its reactive metabolites, (viii) CBZ can directly interact with intact HLA-B*1502/peptide complex, (ix) the N63, I95, and L156 of HLA-B*1502 are the key residues for CBZ binding, and (x) restricted TCRs participate in CBZ-induced SJS/TEN.
From these observations, it is suggested that CBZ non-covalently locates on the surface of HLA-B*1502/peptide complex and is directly touched by TCR, supporting the p:i mechanism involved in CBZ-induced SJS/TEN. This is different from abacavir (ABC)-induced HSR — another HLA participating ADR which was observed to strongly associate with HLA-B*5701 allele (OR = 117, 95% CI: 29–481) (Mallal et al., 2002). Recently, the high-resolution crystal structures of ABC in complex with HLA-B*5701 and self-peptides were solved (Illing et al., 2012; Ostrov et al., 2012), from which it is revealed that the ABC molecule is rooted on the bottom of HLA-B*5701’s peptide-binding groove, reshaping the geometrical property and chemical environment of the antigen-binding cleft, thereby altering the peptide repertoire presented by HLA-B*57:01. The significant difference in the interaction manner of CBZ with HLA-B*1502 and ABC with HLA-B*5701 imparts distinct molecular mechanisms underlying CBZ-induced SJS/TEN and ABC-induced HSR, which is schematically illustrated in Figure 1. As can be seen, the ABC locates beneath antigen peptide and is separated from TCR by the peptide, while CBZ bound at the interface between HLA-B*1502/peptide and TCR. The model can readily explain many experimental observations. For example, the CBZ-specific TCR types are restricted (Ko et al., 2011), whereas broadly polyclonal TCR usage was observed in response to ABC (Illing et al., 2012); ABC alters HLA peptide repertoire (Norcross et al., 2012), but CBZ does not (Yang et al., 2007); the washing procedure can abolish the cytotoxicity elicited by CBZ (Wei, Chung, Huang, Chen, & Hung, 2012) rather than by ABC (Chessman et al., 2008).

### 3.2. Setup of HLA-B*1502/peptide/CBZ complex structure model

First, we tried to construct the structure model of CBZ interaction with HLA-B*1502/peptide complex, where TCR was not present. Because the HLA-B*1502 crystal structure is currently unavailable in the Protein Database Bank (PDB) (Berman et al., 2000), we employed a computational protocol to model its complex structure with cognate peptide. The high-resolution crystal structure (solved at 1.79 Å) of HLA-B*1501 bound with a non-peptide ILGPGSVY was retrieved from the PDB database under the accession ID: 1XR9, which was used as template to generate HLA-B*1502/peptide complex structure models.

The HLA-B*1501 and HLA-B*1502 are highly conserved in their primary sequence and advanced structure. Considering that there are only five residue differences between the two alleles, i.e., E63 N, T94I, L95I, H113Y, and W156L from the former to the latter (the sequence alignment between HLA-B*1501 and HLA-B*1502 is shown in Supplementary Figure S1), instead of the sophisticated homologous modeling method that are widely used to predict protein structure from

![Figure 1](image-url)
homologous crystal template (Martí-Renom et al., 2000), we herein employed virtual site-directed mutagenesis strategy to model the HLA-B*1502 structure based on the HLA-B*1501 template. In the procedure, the mutations E63 N, T94I, L95I, H113Y, and W156L were addressed directly on the crystal structure of HLA-B*1501 to obtain the preliminary structure of HLA-B*1502 complexed with its non-cognate peptide ILGPPGSVY using the SCWRL method (Krivov et al., 2009). Then, the peptide was automatically mutated to several HLA-B*1502 binders separately, including SLFVSNHAY, ELWKNPTAF, NVIRDAVTY, QLGPGGAY, FLFDGSPTY, and SVKPASSSF; these HLA-B*1502-cognate peptides were used previously by Wei et al. (2012) to investigate CBZ-elicited cytotoxic activity. The modeled HLA-B*1502/peptide systems were relaxed by MM minimization and MD simulation to eliminate potential atomic collisions and bond distortions involved in the artifacts.

Subsequently, computational solvent mapping algorithm (Ngan et al., 2012) was employed to detect CBZ-binding hot spots on the surface of HLA-B*1502/peptide complex. The mapping rendered 9 hot spots; most of them located on the region nearby conserved α3

**Figure 2.** CBZ-binding hot spots on the surface of HLA-B*1502/SLFVSNHAY complex detected by computational solvent mapping (Ngan et al., 2012).

Note: The hot spots are marked in different colors.

**Figure 3.** The modeled structure of HLA-B*1502/SLFVSNHAY/CBZ complex. The three key residues N63, I95, and L156 of HLA-B*1502 are highlighted in Ball-and-Stick style.
domain and β2 microglobulin that cannot contribute to the HLA-B*1502–CBZ association specificity, and only three hot spots are close to the B and C pockets of the variable antigen-binding cleft (Figure 2). Therefore, we set a box that fully covers the three effective hot spots and carried out molecular docking calculations to predict the binding modes of CBZ in the box. Consequently, eight clusters of docking-generated modes were obtained, and we performed 100-ns MD simulations for each of the clusters in order to reach an equilibrated state for the HLA-B*1502/peptide/CBZ system. After the MD simulations, two clusters achieved stable form while other six were considerably unstable that the CBZ molecule cannot maintain complex with the HLA-B*1502/peptide. One of the two stable complex structures is shown in Figure 3; it is seen that the CBZ occupies on the surface of the complex, exhibiting a weak binding behavior toward the HLA-B*1502/peptide. MM-PB/SA analysis unraveled that the CBZ interaction energies with HLA-B*1502/peptide in the two stable complexes are separately $-13.4$ and $-15.1$ kcal/mol, which are significantly lower than those of small-molecule drugs bound into the narrow cleft or pocket in their cognate targets. Further, we changed the peptide and repeated above procedure, and consequently, a similar result was obtained, that is, most docking clusters cannot maintain in a tightly bound state during MD equilibration, and in few cases the CBZ only showed a low affinity to HLA-B*1502/peptide, suggesting that the direct binding of CBZ to HLA-B*1502/peptide is unstable that may need additional component such as TCR to stabilize the three-body system.

### 3.3. Addition of TCR to the HLA-B*1502/peptide/CBZ system

From above computational modeling analysis, we found that the CBZ cannot bind tightly to the HLA-B*1502/peptide. It is known that small-molecule drugs are commonly bound within the narrow cleft and deep cave of their protein targets, such as enzyme active site. However, the HLA-B*1502/peptide complex has only a large, flat surface that may not be effective to hold CBZ molecule steadily. In fact, some experimental observations also support this point. For example, no

![Figure 4](image-url)  
**Figure 4.** The HLA-B*1502/peptide/CBZ complexes have not yet been successfully crystallized to date, and a washing procedure can readily abolish CBZ-elicited cytotoxic effect. Thus, it is implied that the presence of TCR might be the prerequisite for forming stable reactive system.
HLA-B*1502/peptide/CBZ complexes have been successfully crystallized to date and a simple washing procedure can abolish CBZ-elicited cytotoxic effect. Thus, it is implied that TCR may play a critical role in stabilizing the system (Figure 4).

Further, we attempted to model the complete HLA-B*1502/peptide/CBZ/TCR complex structure. A computational procedure shown in Figure 5 was used to fulfill the modeling, that is, the HLA-B*1502/peptide/CBZ complex modeled above was superposed onto the HLA-B*3501/peptide/TCR crystal structure (PDB ID: 3MV7) by means of least-squares fitting algorithm (Martin, 1982). The peptide bound to HLA-B*3501 is a 11-mer sequence that forms a protrudent loop that

Figure 5. The procedure used to obtain artificial HLA-B*1502/peptide/CBZ/TCR system. The modeled structure of HLA-B*1502/peptide/CBZ complex and the crystal structure of HLA-B*1502/peptide/CBZ/TCR complex are colored in green and pink, respectively.

Figure 6. Superposition of modeled HLA-B*1502/peptide/CBZ/TCR complex onto crystal HLA-B*3501/peptide/TCR complex (TCR is not shown).
occupies at the pocket region between the two chains of TCR. Instead, HLA-B*1502 is complexed with a 9-mer peptide that exhibits an extended conformation within the peptide-binding cleft of HLA-B*1502. It is evident in Figure 6 that the CBZ-binding site on the surface of HLA-B*1502/9-mer peptide complex corresponds to the protrudent loop of the 11-mer peptide in complex with HLA-B*3501, suggesting that the CBZ molecule in HLA-B*1502/peptide/CBZ system plays a role similar to that of the peptide protrudent loop in HLA-B*3501/peptide/TCR system, which can facilitate the proper recognition of HLA-B*1502/peptide by TCR. Next, the HLA-B*3501 and peptide in original crystal structure were manually removed from the superposition, resulting in an artificial HLA-B*1502/peptide/CBZ/TCR system. Recently, Ko et al. observed a shared and restricted TCR usage in the T-cell clones expanded from CBZ-induced SJS/TEN patients; sequencing of TCR CDR3 regions found that few particular sequence patterns such as VA-22-FISGTY and VB-11-ISGSY are frequently used by CBZ-specific T cells (Ko et al., 2011). In this respect, we adopted different combinations of the CBZ-specific CDR3 sequences to correct TCR CDR3 in the artificial HLA-B*1502/peptide/CBZ/TCR system obtained above. The sequence combinations are extracted from Ko et al. (2011) and tabulated in Table 1. The SCWRL algorithm was employed to mutate the original TCR CDR3 regions, and then the mutated HLA-B*1502/peptide/CBZ/TCR system was equilibrated with 100-ns MD simulation.

Table 1. The specific sequence patterns that were used to define the CD3 regions of TCR (Ko et al., 2011).

| VA CDR3       | VB CDR3           |
|---------------|-------------------|
| CAFISGTYKYIF  | CASSISGYNQSSF     |
| CALMLSQQGSEKLVF | CASSISGYNQERFF   |
| CALSSSSGGYQQKTF  | CASSISGYNQQFF    |
| CALSYQGGKKLF   | CASAHEQYF        |
| CALSDRHFGNEKLTIF | CASSEDRESPYEQQYF |
| CASEVIATFNGEKLTF | CASSISGYSNSFF   |
| CALSEGTFGFCTIF  | CASSISGYNQSF     |
| CALSSSSGGYQQKVTIF | CASSGLAVDNQSSF   |

Figure 7. (A) The modeled HLA-B*1502/peptide/CBZ/TCR complex architecture. (B) Top view of the binding mode of CBZ to HLA-B*1502/peptide complex. (C) Top view of the binding mode of CBZ to TCR.
The resulting HLA-B*1502/peptide/CBZ/TCR complex architecture is shown in Figure 7. The system is stable and can maintain in bound state over the MD simulation procedure. According to the model, CBZ is located at the interface between the HLA-B*1502/peptide complex and TCR (Figure 7(A)), directly contacts the P3–P6 residues of antigen peptide (Figure 7(C)), and bound tightly into the pocket formed by two TCR CDR3 fingers (Figure 7(B)). It is worth noting that the surface of HLA-B*1502/peptide complex has no typical cleft to ‘catch’ CBZ, whereas the deep pocket in TCR appears to well accommodate the CBZ, suggesting a preferred interaction of the ligand with TCR relative to HLA. To gain a quantitative picture, we performed MM-PB/SA analysis to calculate the binding free energies (affinities) $\Delta G_{\text{tot}}$ of CBZ separately to HLA-B*1502/peptide and to TCR over the thousands of snapshots extracted from the MD trajectory of complete system. The obtained results are shown in Figure 8(A), where the peptide is SLFVSNHAY and the two TCR CDR3 fingers adopt different combinations of CBZ-specific sequence patterns. It is evident that the $\Delta G_{\text{tot}}$ value upon the binding of CBZ to TCR is much higher than those to HLA-B*1502/peptide; the former is larger than $-25$ kcal/mol, while the latter is only about $-10$ to $-15$ kcal/mol. We demonstrated that peptide categories have only a modest influence on CBZ affinity to HLA-B*1502/peptide, varying from $-9.7$ kcal/mol (QLGPVGGVF) to $-14.1$ kcal/mol (SVKPASSSF) (Figure 8(B)). In addition, we also investigated the interaction between HLA-B*1502/peptide complex and TCR in the presence or absence of CBZ. It is seen from Figure 8(C) that in four of five examined cases, presence of CBZ can enhance TCR interaction potency with HLA-B*1502/peptide, indicating that the CBZ is an effective adjustor to mediate the recognition of HLA-B*1502/peptide complex by its specific TCR.

3.4. Hypotheses and implications

From the structure model as well as dynamics simulations and energetic analysis, we herein proposed two hypotheses and discussed their biological implications.

Hypothesis 1. The CBZ is first bound to TCR to form TCR/CBZ complex, and then the complex binds HLA/peptide, resulting in the complete HLA/peptide/CBZ/TCR system.

Figure 8. (A) The CBZ affinities separately to HLA-B*1502/peptide complex and TCR with different combinations of CDR3 sequence patterns. (B) The CBZ affinities to HLA-B*1502/peptide complex with different peptide categories. (C) The interaction potencies between the HLA-B*1502/peptide complex and TCR in the presence or absence of CBZ.

Figure 9. The CBZ is first bound to TCR to form TCR/CBZ complex, and then the complex binds HLA/peptide, resulting in the complete HLA/peptide/CBZ/TCR system.
recognizes and binds HLA/peptide presented on cell surface, finally giving rise to the complete HLA/peptide/CBZ/TCR system (Figure 9). According to MM-PB/SA analysis, the binding energy of CBZ to TCR is considerably larger than that of CBZ to HLA-B*1502/peptide (Figure 8(A)), which means that in solvent condition, CBZ can only weakly interact with HLA-B*1502/peptide, maintaining a dynamic balance between bound and unbound states; the presence of TCR can effectively promote the balance shifting into bound state. A direct experimental evidence for this hypothesis is that a simple washing procedure before performing T-cell cytotoxicity assay can completely abolish the cytotoxicity (Wei et al., 2012). Another indirect support is no report for successfully crystallizing the HLA-B*1502/peptide/CBZ three-body system up to now, and we therefore suggest that the crystallization efforts would be attempted in the presence of appropriate TCR subtypes.

Hypothesis 2. Binding of CBZ to TCR shifts TCR specificity; the CBZ-modified TCR may recognize self-peptides presented by HLA-B*1502, and then cause autoimmune reaction (Figure 10). The T-cell thymocytes mature in thymus, where they undergo negative selection that removes portion of the thymocytes with particular TCR subtypes capable of strongly binding with self-peptides presented by medullary thymic epithelial cells (mTECs). In the procedure, only those incapable of recognizing and binding self-peptides can survive and be released to lymph circulation. However, some survived T-cells present particular TCR subtypes with high affinity for CBZ, resulting in CBZ-modified TCR that retrieve the capability of recognizing self-peptides, thereby activating self-reactive cytotoxic T lymphocyte (CTL).

4. Concluding remarks

Although intensive efforts have been addressed on epidemiological investigation of ADRs over the past decades, the molecular mechanism and biological implication underlying ADRs still remain largely unexplored to date. In this study, we attempted to computationally model the first three-dimensional structure of complete HLA-B*1502/peptide/CBZ/TCR complex, regarding a deep understanding of the molecular mechanism and structural basis of CBZ-induced SJS/TEN. The model can be used to explain most observations in previous experiments, according to which the CBZ is located at the interface between the HLA-B*1502/peptide and TCR, directly contacts the antigen peptide, and bound within TCR pocket. We demonstrated that the CBZ can bind TCR more tightly than HLA-B*1502/peptide, suggesting a crucial role of TCR in stabilizing the complex architecture. We also proposed two hypotheses that could be used to guide next wet-lab experiments. This study would help to establish a different molecular framework for CBZ-induced SJS/TEN from that recently published for ABC-induced HSR (Illing et al., 2012; Ostrov et al., 2012).

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2015.1092476.

Disclosure statement

No potential conflict of interest was reported by the authors.
Funding
This work was supported by the National Natural Science Foundation of China [grant number 31200993]; the Science and Technology Project of Sichuan Province [grant number 2015JY0252]; the Young Teacher Doctoral Discipline Fund of Ministry of Education of China [grant number 2012018120025]; and the New Academic Researcher Award of UESTC.

References
Arfeen, M., Patel, R., Khan, T., & Bharatam, P. V. (in press). Molecular dynamics simulation studies of GSK-3β ATP competitive inhibitors: Understanding the factors contributing to selectivity. Journal of Biomolecular Structure and Dynamics. doi:10.1080/07391102.2015.1063457

Balasco, N., Barone, D., & Vitagliano, L. (2015). Structural conversion of the transformer protein RfA:H: New insights derived from protein structure prediction and molecular dynamics simulations. Journal of Biomolecular Structure and Dynamics, 33, 2173–2179. doi:10.1080/07391102.2014.994188

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., … Bourne, P. E. (2000). The protein data bank. Nucleic Acids Research, 28, 235–242. doi:10.1093/nar/28.1.235

Besler, B. H., Merz, K. M., Jr., & Kollman, P. A. (1990). Atomic charges derived from semiempirical methods. Journal of Computational Chemistry, 11, 431–439. doi:10.1002/jcc.540111004

Bharadwaj, M., Illing, P., & Kostenko, L. (2010). Personalized medicine for HLA-associated drug-hypersensitivity reactions. Personalized Medicine, 7, 495–516. doi:10.2217/pme.10.46

Bharadwaj, M., Illing, P., Theodossis, A., Purcell, A. W., Rossjohn, J., & McCluskey, J. (2012). Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. Annual Review of Pharmacology and Toxicology, 52, 401–431. doi:10.1146/annurev-pharmtox-010611-134701

Bond, C. A., & Raehl, C. L. (2006). Adverse drug reactions in United States hospitals. Pharmacotherapy, 26, 601–608. doi:10.1592/phco.26.5.601

Case, D. A., Cheatham, T. E., Darden, T., Gohlke, H., Luo, R., Merz, K. M., … Woods, R. J. (2005). The Amber biomolecular simulation programs. Journal of Computational Chemistry, 26, 1668–1688. doi:10.1002/jcc.20290

Chen, P., Lin, J. J., Lu, C. S., Ong, C. T., Hsieh, P. F., Yang, C. C., … Shen, C. Y (2011). Carbamazepine-induced toxic effects and HLA-B*1502 screening in Taiwan. New England Journal of Medicine, 364, 1126–1133. doi:10.1056/nejmoa1009717

Chessman, D., Kostenko, L., Lethborg, T., Purcell, A. W., Williamson, N. A., Chen, Z., … McCluskey, J. (2008). Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity. Immunity, 28, 822–832. doi:10.1016/j.immuni.2008.04.020

Chung, W. H., & Hung, S. I. (2012). Recent advances in the genetics and immunology of Stevens-Johnson syndrome and toxic epidermal necrosis. Journal of Dermatological Science, 66, 190–196. doi:10.1016/j.jdermsci.2012.04.002

Chung, W. H., Hung, S. I., Hong, H. S., Hsih, M. S., Yang, L. C., Ho, H. C., … Chen, Y. T. (2004). Medical genetics: A marker for Stevens–Johnson syndrome. Nature, 428, 486. doi:10.1038/428486a

Dally, A. K., Donaldson, P. T., Bhatnagar, P., Shen, Y., Pe’er, I., Floratos, A., … Day, C. P. (2009). HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to fluclucoxacin. Nature Genetics, 41, 816–819. doi:10.1038/ng.379

Darden, T., York, D., & Pedersen, L. (1993). Particle mesh Ewald: An N-log(N) method for Ewald sums in large systems. The Journal of Chemical Physics, 98, 10089–10092. doi:10.1063/1.464397

Fan, J. R., Zheng, Q. C., Cui, Y. L., Li, W. K., & Zhang, H. X. (2015) Investigation of ligand selectivity in CYP3A7 by molecular dynamics simulations. Journal of Biomolecular Structure and Dynamics, 33, 2360–2367. doi:10.1080/07391102.2015.1054884

Gastegger, J., & Marsili, M. (1996). Iterative partial equalization of orbital electronegativity – A rapid access to atomic charges. Tetrahedron, 36, 3219–3228. doi:10.1016/0040-4020(80)80168-2

Halgren, T. A. (1996). Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. Journal of Computational Chemistry, 17, 490–519. doi:10.1002/jcc.1096-987x(199604)17

Hsing, S. I., Chung, W. H., Lee, W. R., … Chen, Y. T. (2006). Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. Pharmacogenetics and Genomics, 16, 297–306. doi:10.1097/fpc.0000199500.46842.4a

Hung, S. I., Chung, W. H., Hou, L. B., Chu, C. C., Lin, M., Huang, H. P., … Chen, Y. T. (2005). HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proceedings of the National Academy of Sciences, 102, 4134–4139. doi:10.1073/pnas.0409501012

Illing, P. T., Vivian, J. P., Dudek, N. L., Kostenko, L., Chen, Z., Bharadwaj, M., … McCluskey, J. (2012). Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. Nature, 486, 554–558. doi:10.1038/nature11147

Illing, P. T., Vivian, J. P., Purcell, A. W., Rossjohn, J., & McCluskey, J. (2013). Human leukocyte antigen-associated drug hypersensitivity. Current Opinion in Immunology, 25, 81–89. doi:10.1016/j.coi.2012.10.002

Jacket, A. T., Jack, D. B., & Bayly, C. I. (2002). Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. Journal of Computational Chemistry, 23, 1623–1641. doi:10.1002/jcc.10128

Johnson, J. A., & Bootman, J. L. (1995). Drug-related morbidity and mortality. Archives of Internal Medicine, 155, 1949–1956. doi:10.1001/archinte.155.18.1949

Knapp, B., Omasits, U., & Schreiner, W. (2008). Side chain substitution benchmark for peptide/MHC interaction. Protein Science, 17, 977–982. doi:10.1100/ps.073402508

Ko, T. M., Chung, W. H., Wei, C. Y., Shih, H. Y., Chen, J. K., Lin, C. H., … Hung, S. I. (2011). Shared and restricted T-cell receptor use is crucial for carbamazepine-induced Stevens-Johnson syndrome. Journal of Allergy and Clinical Immunology, 128, 1266–1276. doi:10.1016/j.jaci.2011.08.013

Kollman, P. A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., … Cheatham, T. E. (2000). Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. Accounts of Chemical Research, 33, 889–897. doi:10.1021/ar000035j
Structural modeling of HLA-B*1502/peptide/carbamazepine/T-cell receptor complex architecture

Krivov, G. V., Shapovalov, M. V., & Dunbrack, R. L., Jr. (2009). Improved prediction of protein side-chain conformations with SCWRL4. *Proteins: Structure, Function, and Bioinformatics*, 77, 778–795. doi:10.1002/prot.22488

Mallal, S., Nolan, D., Witt, C., Masel, G., Martin, A. M., Moore, C., … Christiansen, F. T. (2002). Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *The Lancet*, 359, 727–732. doi:10.1016/s0140-6736(02)07873-x

Martin, A. C. R. (1982). Rapid comparison of protein structures. *Acta Crystallographica Section A*, 38, 871–873. doi:10.1107/s056773948201806

Martí-Renom, M. A., Stuart, A. C., Fiser, A., Sánchez, R., Melo, F., & Šali, A. (2000). Comparative protein structure modeling of genes and genomes. *Annual Review of Biophysics and Biomolecular Structure*, 29, 291–325. doi:10.1146/annurev.biophys.29.1.291

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30, 2785–2791. doi:10.1002/jcc.21256

Ngan, C. H., Bohnuud, T., Mottarella, S. E., Beglov, D., Villar, E. A., Hall, D. R., … Vajda, S. (2012). FTMAP: Extended protein mapping with user-selected probe molecules. *Nucleic Acids Research*, 40, W271–W275. doi:10.1093/nar/gks441

Norcross, M. A., Luo, S., Lu, L., Boyne, M. T., Gomarteli, M., Rennels, A. D., … Buchli, R. (2012). Abacavir induces loading of novel self-peptides into HLA-B*57. *AIDS*, 26, F21–F29. doi:10.1097/01.qad.0000423835.668f

Ostrov, D. A., Grant, B. J., Pompeu, Y. A., Sidney, J., Harnhall, M., Southwood, S., … Peter, B. (2012). Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proceedings of the National Academy of Sciences*, 109, 9959–9964. doi:10.1073/pnas.1207934109

Pirmohamed, M., Breakenridge, A. M., Kitteringham, N. R., & Park, B. K. (1998). Fortnightly review: Adverse drug reactions. *BMJ*, 316, 1295–1298. doi:10.1136/bmj.316.7140.1295

Pirmohamed, M., Naisbit, D. J., Gordon, F., & Park, B. K. (2002). The danger hypothesis – Potential role in idiosyncratic drug reactions. *Toxicology*, 181–182, 55–63. doi:10.1016/s0300-483x(02)00255-x

Pompeu, Y. A., Stewart, J. D., Mallal, S., Phillips, E., Peters, B., & Ostrov, D. A. (2012). The structural basis of HLA-associated drug hypersensitivity syndromes. *Immunological Reviews*, 250, 158–166. doi:10.1111/j.1600-065x.2012.01163.x

Rocchia, W., Alexov, E., & Honig, B. (2001). Extending the applicability of the nonlinear Poisson-Boltzmann Equation: Multiple dielectric constants and multivalent ions. *The Journal of Physical Chemistry B*, 105, 6507–6514. doi:10.1021/jp010454y

Ryckaert, J., Cicotti, G., & Berendsen, H. J. C. (1977). Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *Journal of Computational Physics*, 23, 327–341. doi:10.1016/0021-9991(77)90098-5

Sitkoff, D., Sharp, K. A., & Honig, B. (1994). Accurate calculation of hydration free-energies using macroscopic solvent models. *The Journal of Physical Chemistry*, 98, 1978–1988. doi:10.1021/j100058a043

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31, 455–461. doi:10.1002/jcc.21334

Wang, J., Wang, W., Kollman, P. A., & Case, D. A. (2006). Automatic atom type and bond type perception in molecular mechanical calculations. *Journal of Molecular Graphics and Modelling*, 25, 247–260. doi:10.1016/j.jmgm.2005.12.005

Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A., & Case, D. A. (2004). Development and testing of a general amber force field. *Journal of Computational Chemistry*, 25, 1157–1174. doi:10.1002/jcc.20035

Wei, C. Y., Chung, W. H., Huang, H. W., Chen, Y. T., & Hung, S. I. (2012). Direct interaction between HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson syndrome. *Journal of Allergy and Clinical Immunology*, 129, 1562–1569. doi:10.1016/j.jaci.2011.12.990

Wu, X. W., & Brooks, B. R. (2003). Self-guided Langevin dynamics simulation method. *Chemical Physics Letters*, 381, 512–518. doi:10.1016/j.cplett.2003.10.013

Yang, C. W., Hung, S. I., Juo, C. G., Lin, Y. P., Fang, W. H., Lu, I. H., … Chen, Y. T. (2007). HLA-B*1502–bound peptides: Implications for the pathogenesis of carbamazepine-induced Stevens-Johnson syndrome. *Journal of Allergy and Clinical Immunology*, 120, 870–877. doi:10.1016/j.jaci.2007.06.017

Yang, C., Wang, C., Zhang, S., Huang, J., & Zhou, P. (2015). Structural and energetic insights into the intermolecular interaction among human leukocyte antigens, clinical hypersensitive drugs and antigenic peptides. *Molecular Simulation*, 41, 7418–7751. doi:10.1080/08927022.2014.929127

Zhou, P., Huang, J., & Tian, F. (2012). Specific noncovalent interactions at protein-ligand interface: Implications for rational drug design. *Current Medicinal Chemistry*, 19, 226–238. doi:10.2174/092986712803414150

Zhou, P., Wang, C., Ren, Y., Yang, C., & Tian, F. (2013). Computational peptidology: A new and promising approach to therapeutic peptide design. *Current Medicinal Chemistry*, 20, 1985–1996. doi:10.2174/092986712803414150

Zhou, P., Yang, C., Ren, Y., Wang, C., & Tian, F. (2013). What are the ideal properties for functional food peptides with antihypertensive effect? A computational peptidology approach. *Food Chemistry*, 141, 2967–2973. doi:10.1016/j.foodchem.2013.05.140