

**Review Article**

**Anti-epileptic drugs and severe cutaneous drug reactions in certain ethnic populations & HLA association**

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**Abstract**

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe adverse reactions, which could be induced by a variety of drugs. It was proposed that human leukocyte antigen (HLA)-restricted presentation of antigens (drugs or their metabolites) to T lymphocytes initiates the immune reactions of SJS-TEN. The genetic susceptibility and the exact immunological pathogenesis were not clear until the recent studies.

It was first identified that HLA-B\*1502 is strongly associated with carbamazepine (CBZ)-induced SJS-TEN and HLA-B\*5801 with allopurinol-SJS-TEN in Han Chinese. The same associations had been validated across different human populations. For the downstream danger signals, Fas-Fas ligand (FasL) and perforin-granzyme B had been advocated as cytotoxic mediators for keratinocyte death in SJS-TEN. However, expression levels of these cytotoxic proteins from the skin lesions were too low to explain the distinct and extensive epidermal necrosis.

In recent study it was identified that the granulysin, a cytotoxic protein released from cytotoxic T cells or natural killer (NK) cells, is a key mediator for disseminated keratinocyte death in SJS-TEN. The article aims to provide an outlay of both of the genomic and immunologic perspectives of SJS-TEN. These studies give us a better understanding of the immune mechanisms, biomarkers for disease prevention and early diagnosis, as well as providing the therapeutic targets for the treatments of SJS-TEN.

**Keywords:** SJS-TEN- Steven Johnson syndrome- Toxic Epidermal Necrolysis, HLA-Human Leucocyte Antigen, CBZ-Carbamazepine

**1. Introduction**

Adverse drug reactions (ADRs) account for 6–7% of all hospital admissions and remain a major clinical problem. Among them, SJS and TEN are two of the most serious and life-threatening cutaneous ADRs and carry a 10–50% mortality rate<sup>1</sup>. Epilepsy is a common neurological disorder that can be detected in any country. The drug treatment of epilepsy is characterized by unpredictability of efficacy, adverse drug reactions and variable optimal doses. Pharmacogenomics is the use of the genetic makeup of an individual to predict drug response, efficacy and potential adverse drug events.

Strong association between HLA B\*1502 and carbamazepine-induced Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) was demonstrated among Han Chinese in 2004.<sup>2</sup> Studies from Europe showed that the HLA B\*1502 is not a universal marker for SJS/TEN, but is ethnicity specific for Asians. Reports across Asia has shown that the prevalence of HLA B\*1502 is high among Han Chinese (5-15%), Malays (12-15%), and Thais (8-27%), but low among Japan, Korea, Sri Lanka, and most ethnic groups in India.

These drugs include lamotrigine (LTG), carbamazepine (CBZ), phenytoin, phenobarbital, and oxcarbazepine are known anti-epileptic drugs to cause cutaneous drug reactions.

Carbamazepine (CBZ) is an antiepileptic drug (AED) which is most often taken as monotherapy for long term treatment of epilepsy. CBZ is among the commonest AED that causes cutaneous adverse reactions (cADRs)<sup>3</sup> which includes the life-threatening Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Both are characterized by a rapidly developing blistering exanthema of purpuric macules and target-like lesions accompanied by mucosal involvement and skin detachment to a varying extent<sup>4</sup>.

## 2. Clinical manifestations of SJS-TEN

Severe adverse cutaneous drug reaction (ACDR), for example SJS and TEN are life-threatening skin reactions to medications. SJS and TEN are classified as the same disease with different spectrums of severity according to the magnitude of epidermal detachment. Early symptoms of the abrupt onset of SJS usually start with fever, sore throat, and malaise, following by rapidly developing blistering exanthema of macules and target-like lesions accompanied mucosal involvement with less than 10% of skin detachment.<sup>4-5</sup> TEN has similar clinical presentations with a more extensive separation of large sheets of epidermis from the dermis (greater than 30%) and a higher mortality rate (30-40%).<sup>5</sup> Maculopapular exanthema and hypersensitive skin syndrome are other spectrum of cutaneous drug reactions. Maculopapular exanthema is characterized by cutaneous fine pink macules and papules, lesions which usually fade within 1-2 weeks following cessation of drug treatment. Hypersensitive skin syndrome is characterized by multi-organ involvement (e.g. hepatitis and nephritis) accompanied by systemic manifestations (e.g. fever, arthralgia, eosinophilia and lymphadenopathy) in addition to skin rashes.

According to the clinical morphology, SJS/ TEN belong to the group of bullous cutaneous adverse drug reactions, whereas Maculopapular exanthema and hypersensitive skin syndrome are non-bullous reactions.<sup>6</sup>

Among drugs used long term, the greatest risk of SJS/TEN are seen in the first 2 months of use. The agents are mostly antiepileptic drugs e.g. CBZ, phenobarbital, phenytoin, valproic acid and others; e.g. oxycam nonsteroidal anti-inflammatory drugs, allopurinol, and corticosteroids.<sup>7-8</sup>

SJS may prove fatal in about 5% of patients; and TEN in as many as 40% of patients. Sepsis and respiratory distress are the most common complications and ultimately the direct causes of death<sup>9</sup>. Patients experiencing SJS-TEN are affected irreversible mucosal damage and the most serious ocular sequelae. Synechiae, corneal ulcers, symblepharon, and xerophthalmia are frequent ocular complications during the progression of SJS-TEN<sup>10</sup>, and photophobia and xerophthalmia are commonly developed eye sequelae affected SJS-TEN survivals lifelong.<sup>11</sup> Other internal organ involvements at mucosal surfaces are also common in SJS-TEN. With the increasing severity, abnormalities in respiratory tract,<sup>12</sup> gastrointestinal tract<sup>13</sup>, liver, and/or kidney are occasionally reported. The histopathology findings show that the significant feature of large portion epidermis separation from dermis is induced by massive keratinocyte apoptosis in SJS and TEN.<sup>14</sup> Although Fas-FasL interaction was previously considered to be the main effector in triggering apoptosis of keratinocytes, evidence suggests that the granulysin1 is the one actually "turns on" apoptosis of keratinocyte.<sup>15</sup>

## 3. Pharmacogenomics of drug hypersensitivity

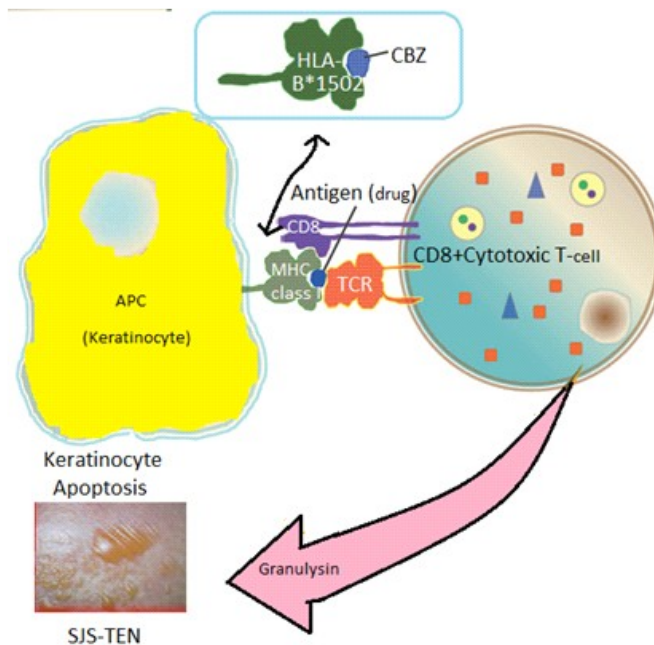
HLA alleles being the main genetic determinants of SJS/TEN was first proposed by Roujeau *et al.*, who reported the weak associations of HLA-A29, B12, and DR7 in sulfonamide-related TEN, and HLA-A2, B12 in oxycam-related TEN in Europeans<sup>16</sup> Following the immunological hypothesis, the most striking evidences of genetic susceptibility to SJS/TEN were provided by our findings that HLA-B\*15:02 is strongly associated with CBZ induced SJS/TEN. The prevalence of HLA B\*1502 is high in some Asian populations. It ranges from 12.0-15.7% in Malay populations from Malaysia and Singapore; 5.7-14.5% in Han Chinese in Taiwan, Hong Kong, Malaysia, Singapore; 8.5-27.5% in Thai, and >10% in Vietnamese. However, the prevalence is low in Sri Lanka, Japan and Korea; similar to prevalence in Europe, American Caucasian and Native and South America. Based on the allele frequency in the U.S. National Marrow Donor Program, similar pattern of HLA-B\*1502 frequency was noted; in which the frequency varies widely among the various ethnic Asians in U.S., and essentially absent among US Caucasians, Hispanics, Native Americans and Africans.<sup>17</sup> The association between CBZ-induced SJS and HLA B\*1502 was extremely high among the Han Chinese in the original<sup>3</sup> and follow-up Taiwan studies, with odd ratio of 2,504 (95% CI 126 to 49,522) and 1,357 (95% CI 193-8,838) respectively. Similar result was also seen among the Han Chinese in Hong Kong.[18] Studies in Malay and Thai populations showed a similar strong association between CBZ-induced SJS and HLA B\*1502, the odd ratio being 16.15 for the Malays in Malaysia.<sup>19-22</sup> However, there was no significant association between HLA B\*1502 and those with CBZ-induced maculopapular eruption and hypersensitivity syndrome, which is probably due to different mechanism from SJS/TEN. Similar association of HLA B\*1502 and CBZ hypersensitivity was not demonstrated in Caucasian population.<sup>23</sup> Other than HLA-B\*1502, HLA-B\*5901 has been

suggested as a candidate marker in CBZ-induced SJS in Japanese with 15.16 relative risk<sup>24</sup>; however, due to its small sample size (10 patients), further investigation is needed to validate this finding. In addition, HLA-A\*0206, was proposed as a marker in SJS-TEN according to the ocular complications in Japanese<sup>25</sup>. It is noted that in addition to HLA-B\*15:02, different members of HLA-B15 family have also been shown to associate with CBZ induced SJS/TEN. HLA-B\*15:02 belongs to HLA-B75. Other members of HLA-B75 (e.g. HLA-B\*15:08, B\*15:11, B\*15:18 and B\*15:21), have been identified in the individuals of CBZ-induced SJS/TEN in different populations. For example, B\*15:08 was identified in the CBZ-induced SJS/TEN patients in Indian.<sup>26</sup> HLA-B\*59:01 was shown to be associated with methazolamide induced SJS/TEN in Korean<sup>27</sup>.

#### 4. Drug-specific, HLA-dependent, T-cell mediated immunity in SJS-TEN

How could small drug molecules induce the dramatic immune reactions, such as SJS/TEN? Multiple factors may be involved in the patho-mechanism of SJS/TEN. As the HLA molecules are the main immune receptors for presenting the foreign antigens, we proposed that in addition to genetic biomarkers, HLA alleles play a pathogenesis role in SJS/TEN. In particular, the highly polymorphic property of HLA molecules among individuals offers a diverse interaction towards different kinds of drug antigens. The specific HLA allele may present a drug/metabolite to the TCRs on the CTLs resulting in cells activation, clonal expansion, and extensive keratinocytes death in SJS/TEN. (Figure-1)

**Figure-1 Keratinocyte apoptosis induced by the immune synapse of drug-HLA-TCR interaction in SJS/TEN**



The proposed pharmaco-immune (p-i) concept<sup>28</sup> is the mainstream explanation for the drug-induced delay cutaneous adverse reactions, suggesting the direct and non-covalent binding between a drug and Tcell receptors (TCR) with HLA molecules takes the responsibility for the drug-induced immunity (Fig. 1).

The pathogenesis of the induction of cytotoxic responses in SJS-TEN is generated by the recognition of offending drugs to HLA class I molecule initiated T-cell activation which results a clonal expansion of CD8+ cytotoxic T-cells in skin.<sup>29</sup> The strong associations between HLA-B\*1502 and CBZ<sup>12</sup> as well as the HLA-B\*5801 and allopurinol, support that drug-induced SJS-TEN is a HLA-restricted immunity.<sup>30</sup> Furthermore, our later results verified 5 peptides showing high affinities for HLA-B\*1502, providing CBZ recognition of HLA, locating at antigen presenting cells<sup>31-32</sup>.

Using the strong HLA-B\*15:02 predisposition in patients with CBZ-induced SJS/TEN as a model, we performed a series of studies to investigate the interaction between drug, peptide, HLA, and TCR in the patho-mechanism of SJS/TEN. We generated stable clones expressing HLA-B\*15:02 molecule and applied liquid chromatography– tandem mass spectrometry to identify CBZ-modified peptides<sup>33,34</sup>. However, no CBZ-modified peptides were detected when we compared the mass spectra of the HLAB\*15:02 bound peptides in the presence or absence of CBZ. By comparison, we found that

endogenous peptides-loaded HLAB\* 15:02 could bind CBZ or its metabolites/analogs directly and present to CTLs (our unpublished data). Recently, we identified shared and restricted TCR usage in CBZ-induced SJS/TEN patients with HLA-B\*15:02 genetic predisposition<sup>35</sup>.

## 5. Cytotoxic signals and immune molecules in SJS/TEN

The central hypothesis to explain the severe mucocutaneous lesions of SJS/TEN is the CTLs/NK cells-mediated immune reactions. Till now, three major classes of cytotoxic proteins, including the Fas–FasL, perforin/granzyme B, and granulysin, are generally advocated for the extensive skin necrosis in SJS/TEN.

### 5.1 Fas–FasL interaction

Although Fas-FasL-induced apoptosis in keratinocytes is one of the most thoroughly studied immune mechanism in SJS-TEN, inconsistent findings of Fas, FasL, and soluble FasL (sFasL) questioned the original hypothesis.<sup>15</sup> Fas was discovered to cause cell death upon binding with its ligand in SJS-TEN. Viard *et al.*<sup>11</sup> suggested the activated Fas servers as a death receptor in triggering apoptosis of keratinocytes in SJS-TEN. The cytoplasmic death domain of Fas undergoes conformational changes upon recognition of FasL. The Fas-FasL then recruits a Fas-associated death domain protein (FADD) which has a affinity to bind to both of the Fas death domain and procaspase 8. Once the procaspase 8 is recruited by FADD, the multiple copies of procaspase 8 are brought together and autoactivate themselves to caspase 8 which triggers the caspase cascade for intracellular DNA degradation.<sup>36</sup>

FasL presented on the cell surface of keratinocytes in TEN patients and sFasL was found to have high levels in the serum, but not in patients with maculopapular drug reaction or normal persons.<sup>37</sup> Metalloproteinases (MPs) present at cell surface in many tissue types, including keratinocytes and cleave FasL into sFasL at its TNF homologous portion.<sup>38,39</sup> The high sFasL serum level observed in TEN patients might cause by the MP cleavage reaction. A study also consistently found an apparent elevation of sFasL within 2 days after the onset of skin damage in a TEN patient.<sup>40</sup> Testing the serological sFasL for a short period at the beginning of onset may help to define the progress of SJS-TEN. The explanations disagreed with either the source of FasL or its role in apoptosis effector. Studies demonstrated that FasL is not located at the extracellular membrane surface of keratinocytes, but rather transporting to the cell surface upon suffering keratinocyte damage.<sup>37,41</sup> Abe *et al.*<sup>42</sup> found that the apoptosis of cultured keratinocytes was induced by adding high levels of sFasL containing sera isolated from a SJS-TEN patient, and was blocked by addition of anti-FasL monoclonal antibody. They also showed the peripheral blood mononuclear cells (PBMCs) of TEN patients were the sites produced high levels of sFasL.

### 5.2 Perforin/granzyme B pathway

High concentrations of granzyme B was found in TEN blister fluid.<sup>43</sup> Nassif A. *et al.*<sup>44</sup> presented a contradictory hypothesis to the Fas-FasL interaction by showing that the cytotoxicity from the blister fluid mononuclear cells in TEN could be blocked by inhibitors of perforin-granzyme B rather than blocked by an anti-Fas monoclonal antibody. Perforin and granzyme B are stored in secretory granules of activated CTLs and NK cells.<sup>45</sup> Perforin binds and punches a channel in the membrane of target cells for entering of the granzyme B to activate the caspase cascade and its following apoptotic pathways.<sup>46</sup>

### 5.3 Other cytokines in SJS-TEN

In addition to Fas-FasL or perforin-granzyme B pathways, some cytokines, including tumor necrosis factor (TNF)- $\alpha$ , interferon (IFN)- $\gamma$ ,<sup>44</sup> and interleukin (IL)-10,<sup>44</sup> were also found to be up-regulated in SJS-TEN. Blister cells of SJS-TEN were reported to secrete IFN- $\gamma$  and stimulate keratinocytes to express TNF- $\alpha$ , FasL, and IL-10 which were present in higher concentrations in the blister fluids as a defense mechanism against CTLs.<sup>44</sup> TNF- $\alpha$  has been reported abundantly presenting in the keratinocytes of epidermis, blister fluid mononuclear cells and macrophages<sup>48,49</sup> and PBMCs<sup>50</sup> in SJS-TEN. TNF- $\alpha$  has been suggested having an up-regulating function in Fas and FasL and via the activation of TNF-receptor 1 (TNF-R1), initiating the downstream FADD and caspases.<sup>51-52</sup> Apoptosis activation in TEN patients had been implied to be induced by TNF.<sup>10</sup> In addition, little evidence showed that TNF- $\alpha$  also can activate TNF-related apoptosis-inducing ligand (TRAIL) and its receptors (TRAIL-Rs), which may result in the activation of FADD apoptotic pathway.<sup>53</sup> Interestingly, contradictory to TNF- $\alpha$  apoptosis inducing role, its binding to TNF-R1 can up-regulate NF- $\kappa$ B<sup>54</sup> and associate with an anti-apoptotic in keratinocytes.<sup>55</sup> Anti-TNF therapy once was thought to be a possible potential treatment for TEN patients; however, no beneficial effect was concluded from anti-TNF by thalidomide in TEN.

## 6. Granulysin is the major factor for keratinocyte apoptosis in SJS-TEN

The 15-kDa granulysin, a cationic cytolytic protein, is secreted extracellularly by CTLs and NK cells via a non-granule exocytotic pathway. The expression level of granulysin rises upon T and NK cell activation. Granulysin has been reported as a serum marker for cell-mediated immunity. Granulysin protein concentrations in the SJS-TEN blister fluids were two to four orders of magnitude higher than perforin, granzyme B or sFasL concentrations, and depleting granulysin reduced the cytotoxicity. (Figure-1)

In addition to having the cytotoxic effect, granulysin also was demonstrated its chemoattractant ability for T lymphocytes, monocytes and other inflammatory cells, and activation function in the expression of a number of cytokines, including RANTES\_CCL5, MCP-1, MCP-3, MIP-1 $\alpha$ /CCL3, IL-10, IL-1, IL-6 and IFN- $\alpha$ .<sup>56</sup> Some mystery regarding SJS-TEN. How does taking a drug lead to secretion of granulysin? How does CD8+T\_NK and NKT cells regulate the secretion of granulysin in SJS-TEN? The specific relationship among offending drugs, HLA alleles, and cytotoxic signals from CLTs\_NK\_NKT cells in SJS-TEN remains further investigation.

## 7. Conclusion

As reviewed in this article, it is known that HLA alleles associated with SJS-TEN may be variable among different human population. These HLA alleles may be not only responsible for the genetic susceptibility, but also play a pathogenesis role, which may present the drugs\_metabolites to CTLs for the initiation of the downstream danger signals in the disease.

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