Update on pathobiology in Stevens-Johnson syndrome and toxic epidermal necrolysis

Shih-Chi Su 1, Wen-Hung Chung 1,2,*

1 Drug Hypersensitivity Clinical and Research Center, Department of Dermatology, Chang Gung Memorial Hospital, Taipei, Keelung, Taiwan
2 School of Medicine, Chang Gung University, Taoyuan, Taipei and Linkou Branches, Taiwan

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A B S T R A C T

Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis (TEN) are rare but life-threatening severe cutaneous adverse reactions (SCARs), which are mainly induced by a variety of drugs. Once considered to be unpredictable, significant progress has been achieved in understanding the pathological mechanisms underlying such reactions. Recent studies suggested that SJS/TEN is a specific immune reaction where human leukocyte antigen (HLA) alleles specific for certain drugs in defined populations are involved in the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Upon the activation, various cytotoxic and immunological signals, including but not limited to Fas/Fas ligand, perforin/granzyme B, and granulysin are launched to mediate the disseminated keratinocyte death in SJS/TEN. This review provides an update on the pathobiology of SJS/TEN in both the genomic and immunologic perspectives. The knowledge gained from these cutting-edge studies will form the basis for better prevention and management of SJS/TEN.

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Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are considered a spectrum of rare but potentially fatal cutaneous diseases, differing only in their extent of skin detachment [with the degree of epidermal detachment less than 10% of body surface area (BSA) being classified as SJS, greater than 30% as TEN, and 10–30% as SJS/TEN overlap] (Figure 1).1 Histopathology typically shows widespread keratinocyte apoptosis and full-thickness epidermal necrosis and detachment with a sparse dermal mononuclear (predominantly T cell) infiltrate.2 Without immediate medical intervention, uncontrolled separation of the epidermis can lead to large denuded areas that cause extreme pain, massive loss of fluid and protein, bleeding, evaporative heat loss with subsequent hypothermia, and infection.1 Although microbial infections have occasionally been reported as the causes of SJS/TEN, this devastating disease is mainly triggered by drug exposure.1,4–6 To date, more than 100 drugs have been associated with SJS and TEN,1 among which aromatic anticonvulsants, sulfonamide antibiotics, allopurinol, oxicam nonsteroidal anti-inflammatory drugs, and nevirapine exhibit a high relative risk.7–10 In this article, we primarily review the current advances in exploring the genetic predisposing factor and pathogenic mechanism of drug-induced SJS/TEN.

Epidemiology

The incidence of SJS/TEN is estimated at one to six cases per million inhabitants per year in Europe and the US.9–13 Yet the mortality rate is 10% for patients with SJS, approximately 30% for patients with SJS/TEN overlap, and almost 50% for patients with TEN.14,15 The condition is more common in adults than in children, and females are affected more frequently than males.13,16 In addition, people over 60 years old seem to be more likely to develop TEN.17,18 This disease occurs sporadically and as a singular event, but the incidence and prevalence can arise in specific populations while exposed to particular drugs. Pharmacogenomic studies have demonstrated that ethnicity and HLA types may predispose patients to SJS/TEN caused by certain drugs, which will be discussed later in this article. Although SJS/TEN has been mainly attributed to drug exposure, approximately 5% of SJS/TEN cases were reported to be associated with HIV infection in Europe.19 Furthermore, other agents such as Mycoplasma pneumonia, Herpes simplex virus, and some neoplastic and autoimmune diseases may have an impact on the incidence of SJS/TEN.5,6,20–24 However, there are still cases of SJS/TEN without any identifiable cause.
Proposed models of drug hypersensitivity

As discussed previously, the majority of SJS/TEN cases were caused by a variety of drugs. It is now recognized that drug-induced SJS and TEN are severe hypersensitivity reactions that involve major histocompatibility class (MHC) I-restricted drug presentation and an expansion of cytotoxic T lymphocytes (CTLs), ultimately leading to extensive keratinocyte death in skin lesions (Figure 2). Because drugs are usually too small to trigger an immunogenic response, several mechanistic models have been proposed to explain how small molecular synthetic compounds are recognized by T cells in an MHC-dependent fashion. These include the hapten/prohapten model, the pei model, and the altered repertoire model.

The hapten/prohapten concept proposes that the drug or its metabolite (hapten/prohapten) reacts with a self-protein through covalent binding to produce a haptenated, de novo product. This product then undergoes antigen processing to generate a novel MHC ligand that is loaded onto the MHC and trafficked to the cell surface, where it activates antigen-specific T lymphocytes. In some cases where drug hypersensitivity takes place rapidly, the immunogenic complexes produced by drug presentation are unlikely to depend on antigen processing or cellular metabolism. A second concept, the p–i or pharmacological interaction with immune receptors model, therefore, is proposed, according to which a noncovalent, labile interaction of the drug with the MHC receptor at the cell surface is involved in MHC-dependent T cell stimulation by various drugs. Neither cellular metabolism nor antigen processing is required in such an interaction. Recently, we identified shared and restricted T cell receptor (TCR) usage in carbamazepine (CBZ)-induced SJS/TEN patients and demonstrated that the endogenous peptide-loaded HLA-B*1502 molecule presented CBZ to CTLs without the involvement of intracellular drug metabolism or antigen processing. These findings favor a role of the p–i model in the pathogenesis of CBZ-induced SJS/TEN.

Another concept, the altered repertoire model, has recently been proposed. In this model, it is postulated that drugs or drug metabolites can bind noncovalently within the pocket of the peptide binding groove of certain MHC molecules with exquisite specificity, allowing a new repertoire of endogenous self-peptides to be bound and presented. Various studies of abacavir-mediated drug hypersensitivity have documented that the binding of abacavir to the antigen-binding cleft of HLA-B*5701 sterically hindered the binding of the original repertoire of peptides, thereby prompting the binding of a new repertoire of peptides bearing immunogenic neoepitopes. In addition, a similar process is also observed in the interaction of CBZ with HLA-B*1502 in SJS/TEN cases. However, this model is insufficient to explain the clinical observation that there are approximately 7% of tolerant CBZ users who carry the predisposing allele, HLA-B*1502, while the drug essentially alters the peptide repertoire in every carrier of the risk allele as proposed. Moreover, about 8% of Han Chinese are carriers of HLA-B*1502, but the prevalence of CBZ-induced SJS/TEN in this population is much lower (< 0.1% of new users). This suggests that additional determinants are implicated in the development of CBZ-mediated SJS/TEN.
Genetic susceptibility to SJS/TEN

Current pharmacogenomic studies have advanced our knowledge on the genetic predispositions to adverse drug reactions. The correlation of HLA alleles with drug-induced SJS/TEN was first reported in cases of sulfonamide- and oximem-related TEN. The physiological role of HLA is to present an antigen to the TCR and subsequently initiate specific T cell-mediated immune responses, which fits perfectly with the pathogenic mechanism of this condition. It is now recognized that genetic associations of HLA alleles with drug hypersensitivity are drug- and ethnicity-specific. We have previously shown that there is a strong association of CBZ-induced SJS/TEN with the HLA-B*1502 allele among Han Chinese in Taiwan. This association was also found in a similar ethnic group of Hong Kong Han Chinese with severe adverse reactions to antiepileptic drugs. Another study further verified the susceptibility of individuals with HLA-B*1502 to CBZ in a Thai population. The US Food and Drug Administration, thus, has recommended genetic test for all patients requiring CBZ whose ancestors have high allele frequency of HLA-B*1502. However, the genetic correlation between HLA-B*1502 and CBZ-induced SJS was relatively weak in Indians and also in Korean, Japanese, and European groups. This discrepancy may be related to different frequencies of this allele among distinct populations.

In addition to the ethnic specificity, the genetic susceptibility to drug-induced SJS/TEN highly depends on the nature of the offending medications. HLA-B*5801, rather than HLA-B*1502, was found to be strongly linked to allopurinol-mediated SJS/TEN. Unlike the cases in CBZ-induced SJS/TEN, the association of HLA-B*5801 with allopurinol-mediated SJS/TEN was not only observed in other southeast Asians but also in Japanese, Korean patients, and patients of European origin.

Moreover, regardless of the ethnic differences in genetic backgrounds and HLA allele frequencies, as well as inconsistent clinical classification of the enrolled cases and sample sizes, other HLA—drug associations that contribute to the pathogenesis of SJS/TEN have been reported; HLA-A*3101 and HLA-B*1511 with CBZ, HLA-B*1502 with phenytoin, HLA-B*38 with sulfamethoxazole or lamotrigine, HLA-B*73 with oximem, and HLA-B*5901 with methazolamide. A summary of genetic relationships between various HLA allotypes and drug-induced SJS/TEN is shown in Table 1 and Figure 3. The knowledge gained from such pharmacogenomic studies is highly beneficial for lowering the incidence of drug-induced SJS/TEN and ultimately generating genetic databases that allow prescriptions to be tailored to an individual’s genetic risk.

Cytotoxic and immunological mediators of drug hypersensitivity in SJS/TEN

With the identification of specific HLA alleles as the predisposing factor to the disease, it becomes clear that the pathogenesis of drug-mediated SJS/TEN involves MHC-restricted activation of CTL response. This CTL response requires several downstream signals or mediators to trigger extensive keratinocyte death. Fas—Fas ligand (FasL) interaction is the first reported mechanism that modulates keratinocyte apoptosis in TEN. In this landmark study, the pathophysiological presence of both Fas and FasL in the epidermis of patients with TEN was unveiled. More specifically, an elevation of soluble FasL (sFasL) and epidermal FasL expression were observed in the sera and skin biopsy specimens from patients with TEN, respectively, implying that sFasL detected in the sera resulted from cleavage of a membrane-bound FasL on the epidermal cells of TEN patients. In addition, apoptosis was abrogated while TEN skin sections were pretreated with a FasL-blocking antibody. The authors concluded that TEN keratinocytes express lytically active FasL, whose ligation with Fas on adjacent keratinocytes leads to apoptosis. A follow-up study further showed that FasL expression was restricted to the basal and suprabasal layers of normal human epidermis. Despite demonstrating the coexpression of both Fas and FasL in the lower dermis, keratinocyte FasL, however, was primarily cytoplasmic in vivo and unable to cause apoptosis. This proposed mechanism was also challenged by others. It is documented that keratinocytes failed to overexpress Fas, Moreover, elevated levels of sFasL in SJS and TEN were detected by the other group, but they found no membrane-bound FasL expression on keratinocytes in TEN patients or in healthy controls. Noteworthy, sFasL levels increased significantly when peripheral blood mononuclear cells (PBMCs) from TEN patients were cultured with the offending drug, suggesting an alternative source of serum sFasL in SJS/TEN. Although the involvement of Fas—FasL interactions in mediating keratinocyte death in SJS/TEN was pointed out in numerous studies, several questions in terms of the origin of sFasL, the coexistence of Fas and FasL in the same areas of epidermis, and a defined role for FasL in TEN apoptosis still remain unanswered.

Another hypothetical mechanism involves perforin and granzyme B, two cytolytic proteins that are released by activated cytotoxic T cells and natural killer (NK) cells. It is believed that perforin creates pores within the cell membranes, through which granzyme B can enter the cell and induce apoptosis by turning on the caspase cascade. However, new evidence indicates that perforin and granzyme B are enclosed in a vesicle and delivered into the cell through the mannose 6-phosphate receptor to cause apoptosis through the cytoplasmic and unable to cause apoptosis. A follow-up study further showed that FasL expression was restricted to the basal and suprabasal layers of normal human epidermis. Despite demonstrating the coexpression of both Fas and FasL in the lower dermis, keratinocyte FasL, however, was primarily cytoplasmic in vivo and unable to cause apoptosis. This proposed mechanism was also challenged by others. It is documented that keratinocytes failed to overexpress Fas, Moreover, elevated levels of sFasL in SJS and TEN were detected by the other group, but they found no membrane-bound FasL expression on keratinocytes in TEN patients or in healthy controls. Noteworthy, sFasL levels increased significantly when peripheral blood mononuclear cells (PBMCs) from TEN patients were cultured with the offending drug, suggesting an alternative source of serum sFasL in SJS/TEN. Although the involvement of Fas—FasL interactions in mediating keratinocyte death in SJS/TEN was pointed out in numerous studies, several questions in terms of the origin of sFasL, the coexistence of Fas and FasL in the same areas of epidermis, and a defined role for FasL in TEN apoptosis still remain unanswered.

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Our recent study of granulysin, which is a multifunctional, cytolytic protein, further provides a revealing insight into the pathogenic mechanism underlying widespread keratinocyte death in SJS/TEN. We have demonstrated that the level of 15-kDa granulysin was predominant over that of perforin, granzyme B, and sFasL in the SJS/TEN blister fluids. Depletion of granulysin reduced the cytotoxicity of SJS/TEN blister fluids to keratinocytes, and a further injection of granulysin into mouse skin resulted in blistering and epidermal necrosis mimicking SJS/TEN. Moreover, an

<table>
<thead>
<tr>
<th>Drug</th>
<th>HLA allele</th>
<th>Ethnic population</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopurinil</td>
<td>B*5801</td>
<td>Han Chinese, Thai, Japanese</td>
<td>40–44</td>
</tr>
<tr>
<td></td>
<td>B*5801</td>
<td>Han Chinese, Thai, Japanese</td>
<td>40–44</td>
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<td>Han Chinese, Thai, Japanese</td>
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<td></td>
<td>B*5801</td>
<td>Han Chinese, Thai, Japanese</td>
<td>40–44</td>
</tr>
<tr>
<td></td>
<td>B*5801</td>
<td>Han Chinese, Japanese</td>
<td>40–44</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>B*1502</td>
<td>Han Chinese, Thai, Indian</td>
<td>34–37</td>
</tr>
<tr>
<td></td>
<td>B*1511</td>
<td>Japanese</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>B*5901</td>
<td>Japanese</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>A*3101</td>
<td>Han Chinese, Japanese, European</td>
<td>46,48,70</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>B<em>1502, B</em>38</td>
<td>Han Chinese</td>
<td>44,49</td>
</tr>
<tr>
<td></td>
<td>B<em>5801, A</em>6801</td>
<td>Han Chinese</td>
<td>44,49</td>
</tr>
<tr>
<td></td>
<td>CW*0718</td>
<td>European</td>
<td>44,49</td>
</tr>
<tr>
<td>Oximem</td>
<td>B<em>73, A</em>2, B*12</td>
<td>European</td>
<td>44,71</td>
</tr>
<tr>
<td></td>
<td>DRB1*1301</td>
<td>Korean, Japanese</td>
<td>50</td>
</tr>
<tr>
<td>Methadrazole</td>
<td>B<em>5901, CW</em>0102</td>
<td>Korean, Japanese</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>B<em>73, A</em>2, B*12</td>
<td>European</td>
<td>44,71</td>
</tr>
<tr>
<td></td>
<td>B*1502</td>
<td>Han Chinese, Thai</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>B*1502</td>
<td>Han Chinese, Thai</td>
<td>49</td>
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<td></td>
<td>B*1502</td>
<td>Han Chinese, Thai</td>
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<td>B*1502</td>
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<td></td>
<td>B*1502</td>
<td>Han Chinese, Thai</td>
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</tr>
<tr>
<td></td>
<td>B*38</td>
<td>European</td>
<td>44,49</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>B*38</td>
<td>European</td>
<td>44</td>
</tr>
</tbody>
</table>

HLA = human leukocyte antigen; SJS/TEN = Stevens-Johnson syndrome/toxic epidermal necrosis.
increase in the serum levels of granulysin during the early stage of SJS/TEN but not in patients with drug-induced maculopapular eruption was also observed by another group. In addition to the cytotoxic effect, granulysin has been shown to serve as a chemotactant for T lymphocytes, NK cells, monocytes, and other inflammatory cells, which may, in part, account for the infiltration of CTLs and NK cells observed in skin lesions of TEN patients. It is expected to have a specific antigranulysin therapy for SJS/TEN in the near future. However, the search for an available effective therapeutic agent based on the pathomechanism of SJS/TEN is urgent before embarking on the long road to developing a new specific anti-granulysin drug.

Other than those mentioned above, various cytokines/chemokines that are responsible for trafficking, proliferation, and activation of T cells and other immune cells have been found to be elevated in the skin lesions, blister fluids, blister cells, PBMCs, or plasma of SJS/TEN patients. These include interferon-gamma (IFN-gamma), TNF-alpha, interleukin-2 (IL-2), IL-5, IL-10, IL-12, IL-13, IL-18, C–C chemokine receptor type 3 (CCR3), C–X–C chemokine receptor 3 (CXCR3), CXCR4, and CCR10.

**Conclusion**

Despite the rareness, SJS and TEN have a substantial impact on public health because of high mortality. The condition frequently results in lasting disability of patients and hinders the physicians from prescribing the medications that are commonly administered in clinical practice. Fortunately, recent studies have made a significant stride in our understanding of the pathobiology of SJS and TEN during the last decade. Major breakthroughs, such as the discovery of genetic markers, the clarification of HLA–drug–TCR interactions, and the identification of granulysin as the key mediator in epidermal necrolysis, offer us a solid foundation for disease prevention and early diagnosis, as well as providing the potential therapeutic target for the treatment of SJS/TEN. The translation of HLA-B*1502 and CBZ-induced SJS/TEN from discovery to a guideline-based test used routinely in Taiwan is a notable example (Table 2). Although the incidence has improved with the successful

**Table 2** Roadmap of contemporary CBZ–SJS research and its clinical applications.

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1998–2004</td>
<td>CBZ, an aromatic anticonvulsant, was recognized as the major cause of SJS, as listed in the records of the Taiwan Drug Relief Foundation.</td>
</tr>
<tr>
<td>2004</td>
<td>A strong association of CBZ-induced SJS with HLA-B*1502 in Han Chinese was first uncovered in Taiwan.</td>
</tr>
<tr>
<td>2006</td>
<td>The correlation of CBZ-induced SJS with HLA-B<em>1502 was not existent in Caucasian patients, indicating an ethnic specificity of HLA-B</em>1502 allele.</td>
</tr>
<tr>
<td>2007–2008</td>
<td>The association of CBZ-induced SJS with HLA-B*1502 was found among many populations in Southeast Asia.</td>
</tr>
<tr>
<td>2007</td>
<td>The US Food and Drug Administration published an alert to healthcare professionals on the use of CBZ in Asians. (The incidence rate of CBZ-induced SJS is 5.9/10,000 in Taiwan and 0.2/10,000 in the US).</td>
</tr>
<tr>
<td>2007</td>
<td>The Taiwan and US Food and Drug Administration relabeled the drug information of CBZ and recommended a genetic screening of HLA-B*1502 in certain Asian groups before the use of CBZ.</td>
</tr>
<tr>
<td>2010</td>
<td>The screening of HLA-B*1502 was approved as a guideline-based test for patients before the first administration of CBZ and covered by the Bureau of National Health Insurance in Taiwan.</td>
</tr>
</tbody>
</table>

CBZ – carbamazepine; HLA – human leukocyte antigen; SJS – Stevens-Johnson syndrome.
application of uncovering genetic markers, treatment for SJS/TEN remains ineffective and only supportive. Further studies on thera-
peutic aspects are needed to develop more options for disease management and to achieve a better outcome.

Acknowledgments
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