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Comprehensive assessment of T cell receptor β repertoire in Stevens–Johnson syndrome/toxic epidermal necrolysis patients using high-throughput sequencing

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ABSTRACT

Stevens–Johnson syndrome (SJS) /toxic epidermal necrolysis (TEN) are life-threatening severe cutaneous adverse drug reactions characterized by widespread epidermal necrosis. Recent studies have indicated that SJS/TEN is a specific immune reaction regulated by T cells. Certain drug serves as foreign antigens that are presented by major histocompatibility complex (MHC) and recognized by T cell receptors (TCRs), inducing adaptive immune responses. However, few studies have performed detailed characterization of TCR repertoire in SJS/TEN, and it remains unclear whether the particular types of TCRs expanded clonally are drug-specific, which would provide a potential underlying mechanism of SJS/TEN. In this study, using high-throughput sequencing, we comprehensively assessed the diversity, composition and molecular characteristics of the TCRβ repertoires in 17 SJS/TEN patients associated with three different causative drugs including methazolamide (MZ), carbamazepine (CBZ) and allopurinol (ALP). Systematic analysis of the TCRβ sequences revealed that SJS/TEN patients had more highly expanded clones and less TCR repertoire diversity, and the TCR repertoire diversity of these patients showed certain associations with the clinical severity of disease. Similar predominant clonotypes, shared-usage TRBV/TRBJ subtypes and combinations thereof were observed among different subjects with the same causative agent. Our observations provide enhanced understanding of the role of T lymphocytes in the pathogenesis of SJS/TEN and enumerate potential therapeutic targets.

1. Introduction

Drug hypersensitivity remains a major clinical problem worldwide, causing considerable costs for health care systems annually. Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but life-threatening severe drug hypersensitivity reactions (Duong et al., 2017). SJS/TEN are characterized by atypical target lesions, blisters and epidermal detachment, and differ only in the extent of epidermal detachment. SJS is defined as epidermal detachment amounting to less than 10% of body surface area (BSA), whereas TEN is defined as an area of detached epidermal greater than 30%. Cases with 10–30% epidermal detachment are referred to as SJS-TEN overlap (Duong et al., 2017). SJS/TEN are predominantly induced by drugs. Currently, more than 100 drugs have been reported to be associated with the inductions of SJS/TEN. The most frequently incriminated drugs are anticonvulsants, allopurinol, antibiotics and non-steroidal anti-inflammatory drugs (Hoetzenecker et al., 2016). Although the incidence of SJS/TEN ranges from 2 to 7 cases/million people per year,

Abbreviations: SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; TCR, T cell receptor; MZ, methazolamide; CBZ, carbamazepine; ALP, allopurinol; NC, normal control; HLA, human leukocyte antigen; CDR3, complementarity-determining region 3

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they can cause multiorgan failure, serious sequelae and significant mortality (Lee et al., 2017; Levi et al., 2009; Mockenhaupt et al., 2008; Sassolas et al., 2010; Sekula et al., 2013). The average reported mortality rate for SJS is between 5% and 10%, and this increases to almost 50% in patients with TEN (Firoz et al., 2012; Schneck et al., 2008; Sekula et al., 2013).

The pathological mechanisms underlying SJS/TEN have not been fully elucidated. Accumulating evidence has shown that SJS/TEN are T-cell regulated delayed-type hypersensitivity reactions, and T cells participate in attack of the skin, leading to extensive keratinocyte death. Histopathological analysis of SJS/TEN lesions shows widespread keratinocyte apoptosis and full-thickness epidermal necrosis and detachment, with numerous T cell infiltrates in the sparse dermis (Paquet et al., 2005). T cells infiltrate the skin lesions and secrete cytotoxic proteins/chemokines or directly contact keratinocytes, resulting in disseminated keratinocyte death in SJS/TEN patients (Su and Chung, 2014). Recently, pharmacogenomic studies have demonstrated a considerable association between certain human leukocyte antigen (HLA) genes and specific drug-induced SJS/TEN in different populations. For example, methazolamide (MZ)-, carbamazepine (CBZ)- and allopurinol (ALP)-induced SJS/TEN have been strongly associated with HLA-B∗59:01, HLA-B∗15:02 and HLA-B∗58:01, respectively (Negrini and Becquemont, 2017). Further studies have verified that the drug or its metabolites presented by specific HLA molecules to T cells induced the activation of drug-specific T cells (Wei et al., 2012; Yun et al., 2014). However, not all risk-associated HLA allele carriers will suffer from SJS/TEN after exposure to causative drugs. The positive predictive value (PPV) of HLA-B*58:01 for ALP induced SJS/TEN(ALP-SJS/TEN) is only 3%, and the PPV of HLA-B*15:02 for CBZ induced SJS/TEN (CBZ-SJS/TEN) is also only 3% (Redwood et al., 2018). This indicates that, in addition to risk-associated HLA alleles, other factors are also crucial in the pathogenesis and development of SJS/TEN.

The T cell receptor (TCR) is a heterodimer of two trans-membrane polypeptide chains and acts as a key player in the immunological synapse. It is specifically involved in antigen recognition and HLA restriction. In general, each T cell encodes a single unique TCR, and more than 90% of mature peripheral T cells express αβTCR. In response to the immense level of foreign antigen, it is critical to develop and maintain a highly diversified TCR repertoire. The theoretical diversity in the αβTCR repertoire is estimated at 1018 in humans (Attaf et al., 2015; Sewell, 2012). The specificity and diversity of TCRs predominantly depend on complementarity-determining region 3 (CDR3), which is encoded by V(D)J recombination and interacts with the antigen presented by the HLA. Given that the CDR3 region is unique to each T cell clone, a specific CDR3 sequence can be used as an indication for a unique T cell clone (Li et al., 2013). The TCR β chain has greater combinatorial and junctional diversity than the α chain due to its D gene component. Therefore, most TCR repertoire studies have focused on the TCR β chain (Woodsworth et al., 2013). Flow cytometric analysis, spectra-typing and real-time PCR analysis have been used to estimate the TCR repertoire. However, these techniques have only analysed the dominant V gene segments and have lacked sufficient depth of coverage and resolution to accurately assess the diversity of the TCR repertoire (Six et al., 2013). With the development of next generation sequencing (NGS) technologies, it has become possible to characterize millions of sequences, which has been proven to be more helpful for analysing the total TCR repertoire (Six et al., 2013). Analysis of the TCR repertoire offers a clearer understanding of both the versatility and diversity of the overall immune T-cell compartment, helping to determine the correlation between TCR usage and disease severity.

Over the last several decades, altered TCR repertoire patterns and specific TCR clones have been demonstrated in a variety of different disorders ranging from immune-associated diseases and infections to malignancies (Attaf et al., 2015; Woodsworth et al., 2013). In SJS/TEN, certain drugs serve as foreign antigens that are presented by HLA and recognized by T cell receptors (TCRs) to induce adaptive immune responses. Ko et al. analysed TCR profiles for cultured CBZ-activated T cells by RT-PCR and identified VB-11-ISGSY as the most predominant clonotype shared among different subjects (Ko et al., 2011). Furthermore, preferential TCR-V-β usage was also found in oxypurinol-activated T-cells from patients with ALP-SJS/TEN (Chung et al., 2015). All studies concerning the association between TCR clonotypes and SJS/TEN have focused on cultured cells in vitro. However, few studies have reported detailed characterization of TCR repertoires in SJS/TEN, and it remains unknown whether clonal expansion of specific TCR clonotypes is the general mechanism for SJS/TEN. In this study, using high-throughput sequencing, we comprehensively assessed the diversity, composition and molecular characteristics of the TCRβ repertoire in 17 SJS/TEN patients associated with three known culprit drugs, including for clonotype frequency, TCR repertoire diversity and V/J gene utilization. These results will contribute to greater understanding of the role of T lymphocytes in the pathogenesis of SJS/TEN and help to explore potential therapeutic targets.

2. Materials and methods

2.1. Subjects

Patients were recruited from the Dermatology or the Emergency ward of Huashan Hospital-affiliated Fudan University. The diagnosis of SJS/TEN was made by two independent dermatologists based on clinical signs and symptoms and was confirmed by skin biopsies showing full-thickness necrosis of the epidermis on pathological examination. Culprit drugs were determined according to the Algorithm for Drug Causality for Epidermal Necrolysis (ALDEN), a causality-assessment tool for discriminating culprit drugs when patients were receiving multiple drugs (Sassolas et al., 2010). Patients had a chronic disease history or other bacterial or viral infections, and patients who had received chemotherapy or other biological treatments in the past five years were excluded from the study. Blood samples were obtained before the patients received glucocorticoid therapy. Normal controls consisted of six healthy subjects with no clinical or laboratory evidence of infectious diseases or immunological disorders. This study was approved by the ethics committee of Huashan Hospital-affiliated Fudan University, and informed consent was obtained from each participant before the start of the study.

2.2. Peripheral blood mononuclear cells (PBMCs) isolation and genomic DNA extraction

PBMCs were isolated from 5 ml fresh EDTA K2 anticoagulant-treated blood by using Ficoll-Paque (GE healthcare-science AB, Uppsala, Sweden) density gradient separation according to the manufacturer's protocol. Then, the purified PBMCs were immediately processed for genomic DNA isolation using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

2.3. Multiplex-PCR amplification of the TCRβ CDR3 region and high-throughput sequencing

TCRβ CDR3 regions were amplified and sequenced by BGI Tech (Shenzhen, China) using previously described protocols (Bai et al., 2015). Briefly, multiplexed PCR amplification was performed to amplify all possible rearranged TCRβ CDR3 sequences from the samples using the genomic DNA as a template, using a panel of primers specific for the TCRβ CDR3 V and J regions. After amplification and agarose gel electrophoresis selection, the products (100–190 bp) were excised and purified using a QIAquick PCR Purification Kit. After end-repair, dA-tailing, adapter ligation and PCR amplification, the specific DNA fragments (200–314 bp) were selected and purified. Finally, the libraries were amplified with cBot to generate the cluster on the flow cell, and the paired-end sequencing of samples was carried out with a read-

length of 100 bp through the use of Illumina Hiseq2000 platform.

2.4. Data processing and analysis

The raw sequencing data were filtered according to four strict criteria: (1) reads contaminated by adapter sequences; (2) reads with more than 5% uncalled bases (N); (3) reads with an average quality score lower than 15 (based on the Illumina 0–41 quality system); and (4) PE reads with low-quality base readings (Q-score < 10) at the ends of reads or short reads (length < 60 bp). After filtration, the high-quality paired-end reads were merged into one contig sequence using FLASH57 software. To identify V, D and J segments, the merged sequences were aligned via miXCR (Bolotin et al., 2015). The CDR3 region was then identified within the sequencing reads according to the definition established by the International Immunogenetics collaboration, which began with the second conserved cysteine at the 3′ portion of Vβ segment and ended with the conserved phenylalanine at the 5′ portion of Jβ segment. The contrasting analysis of main composition characteristics of the TCR repertoire included: highly expanded clone number and sequence, diversity of the TCR repertoire, and usage and pairing of TRBV and TRBJ gene families.

2.5. Statistical analysis

The statistical analyses were conducted with GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Mean values were compared using one-way ANOVA. The nonparametric comparisons between groups were performed using the Mann–Whitney test. P values less than 0.05 were considered significant.

3. Results

3.1. Clinical features of the patients

Seventeen patients were enrolled in the study and categorized into three groups: MZ-induced SJS/TEN (MZ-SJS/TEN), CBZ-SJS/TEN and ALP-SJS/TEN. Among these patients, thirteen were diagnosed with SJS and four with TEN. All patients had oral mucosal involvement, fifteen had ocular mucosal involvement and eleven had genital mucosal involvement. Score of toxic epidermal necrolysis (SCORTEN) is scoring system that evaluates several clinical parameters within 24 h of hospitalization, including age, tachycardia, initial epidermal detachment area, serum urea, serum glucose, and bicarbonate (Bastuji-Garin et al., 2000). The SCORTEN values of the patients ranged from 0-3. The normal controls included six healthy donors. Detailed demographic information is summarized in Table S1.

3.2. Summary of sequence data

To reduce bias caused by HTS, 2 M data was randomly captured for analysis in each sample. The numbers of total clonotypes were 1,648,554 ± 45,003, 1,657,919 ± 39,487, 1,614,991 ± 69,691 and 1,597,848 ± 79,362 in MZ-SJS/TEN, CBZ-SJS/TEN, ALP-SJS/TEN and normal control groups, respectively, without any significant differences (P > 0.05). Detailed sequencing information is shown in Table S2.

3.3. Enrichment of expanded clones in different samples

To evaluate the degree of TCR clonal expansion in each subject, we first summarized the unique clonotype distribution according to their abundance in each subject (Fig. 1). Next, we calculated the number of highly-expanded clones (HECs), which were defined as clones with a frequency greater than 0.5% of the total clones. We found that HECs number increased significantly in SJS/TEN patients compared with healthy controls (MZ-SJS/TEN 8.40 ± 3.87, CBZ-SJS/TEN 8.83 ± 2.64, ALP-SJS/TEN 9.33 ± 3.33, NC 2.67 ± 1.97, all $P < 0.05$, Fig. 2A). There was no significant difference between the different drug-induced SJS/TEN groups. These results indicated that the dominant clonotypes were substantially elevated in SJS/TEN patient repertoires relative to that in healthy donors, which strongly indicated the occurrence of oligoclonal proliferation clones in SJS/TEN patients. We further investigated the sequences of the HECs between the NC and SJS/TEN groups or different individuals of the same group (Table S3). Although the expanded clonotypes differed from case to case, some clonotypes of different samples showed some smaller sequence features or motif in the same drug-induced SJS/TEN groups (Fig S1). In comparison, the HECs of SJS/TEN patients were absent or detected at very low frequencies in normal controls (data not shown).

3.4. TCR repertoire diversity analysis and relationship with disease severity

To quantitatively evaluate the overall TCR repertoire diversity of the patients, we calculated the Shannon–Wiener index (H′) of each sample. In our research, H′ was significantly lower in the three groups of SJS/TEN patients compared with the normal controls (MZ-SJS/TEN: 10.35 ± 0.71, CBZ-SJS/TEN: 10.66 ± 1.13, ALP-SJS/TEN: 10.80 ± 0.94 vs NC: 13.20 ± 0.94, all P < 0.05, Fig. 2B). There was no significant difference between the different drug-induced SJS/TEN groups. These results suggest that TCR repertoire diversity in SJS/TEN patients was less heterogeneous.

We then analysed the relationship between TCR diversity and disease severity. The index evaluating the disease severity included the detached BSA and SCORTEN. H′ of the TEN patients (more than 30% detached BSA) was significantly lower than the SJS patients (less than 10% detached BSA) (9.57 ± 0.72 vs 10.94 ± 0.71, respectively, P < 0.05, Fig. 3A). Regarding the SCORTEN, H′ values of patients with scores of 2–3 were slightly lower than patients with scores of 0–1, although this difference was not statistically significant (10.20 ± 0.66 vs 10.85 ± 0.98, P > 0.05, Fig. 3B).

3.5. TRBV/TRBJ usage and combination analysis

The V and J gene segments are the main components of the TCR. To determine whether drug-related differences existed in TRBV and TRBJ usage of SJS/TEN patients, we analysed the V and J gene segments in each sample. The top 20 V genes and J genes expressed in each sample are listed in Fig. 4A and B. We then compared their usage levels in different drug-induced SJS/TEN patients with normal controls. In MZ-induced SJS/TEN patients, significantly increased TRBV subtypes included TRBV19 and TRBV7-3, whereas significantly decreased TRBV and TRBJ subtypes included TRBV5-4, TRBV7-9, TRBJ2-5 and TRBJ2-6 (P < 0.05, Figs. S2A and S3A). In CBZ-induced SJS/TEN patients, significantly decreased TRBV subtypes included TRBV10-1, TRBV11-1, TRBV11-2, TRBV14, TRBV15, TRBV29-1 and TRBV7-9 (P < 0.05, Figs. S2B and S2B). In ALP-induced SJS/TEN patients, significantly increased TRBV and TRBJ subtypes included TRBV20-1 and TRBJ2-7, whereas significantly decreased TRBV and TRBJ subtypes included TRBV11-3, TRBV15, TRBV27, TRBV6-2, TRBV6-3, TRBJ1-1, TRBJ1- 4 and TRBJ2-6 (P < 0.05, Figs. S2C and S3C).

TRBV/TRBJ combination was an important source of unique CDR3 sequences. To examine the potential contribution of specific TRBV/TRBJ combinations to disease progression, we also compared the relative frequencies of V-J combinations in different drug induced SJS/TEN patients with normal controls. Differential usage of TRBV/TRBJ combinations in each group were found as follows: In MZ-induced SJS/TEN patients, there were 6 increased and 48 decreased TRBV/TRBJ combinations ($P < 0.05$, Fig. 5A), with increased combinations including: TRBV5-6/J1-5, TRBV6-5/J1-3, TRBV6-6/J2-1, TRBV5-8/J1-2, TRBV19/J2-7 and TRBV2/J1-3. In CBZ-induced SJS/TEN patients, there were 3 significantly increased and 57 significantly decreased TRBV/TRBJ combinations ($P < 0.05$, Fig. 5B), with increased combinations including: TRBV20-1/J1-3, TRBV12-3/J2-5 and TRBV6-5/J1-

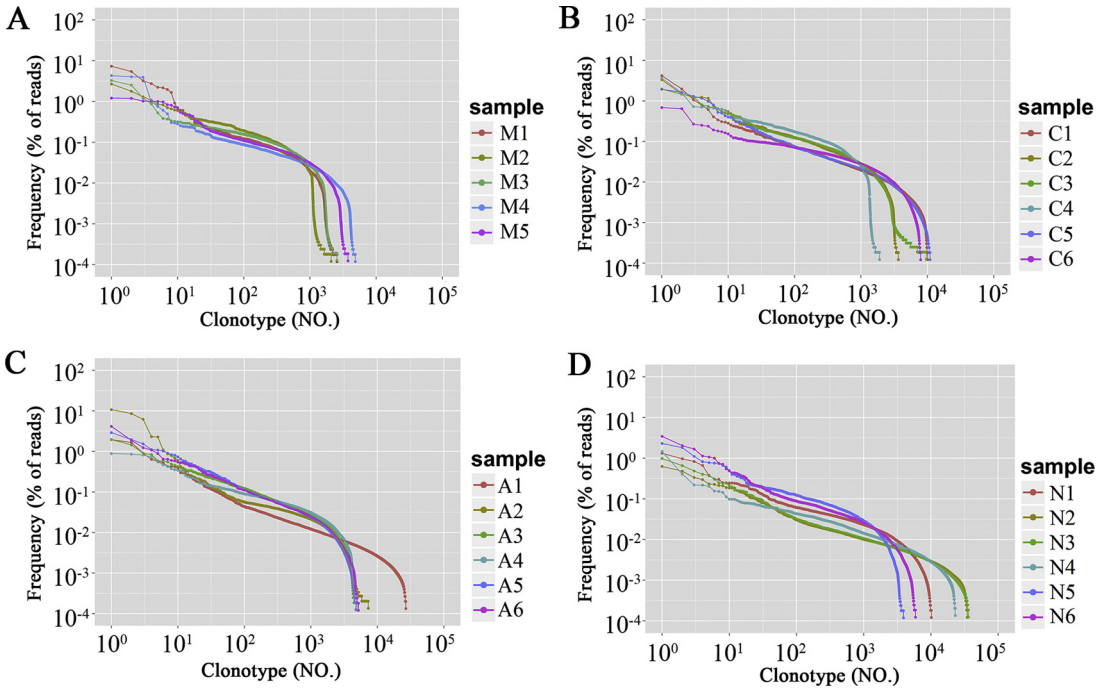

Fig. 1. Unique CDR3 sequence distribution. The X-axis depicts the number of the unique clonotype, and the Y-axis depicts the frequency of each clonotype. (A) MZ-SJS/TEN samples, (B) CBZ-SJS/TEN samples, (C) ALP-SJS/TEN samples, and (D) NC samples.

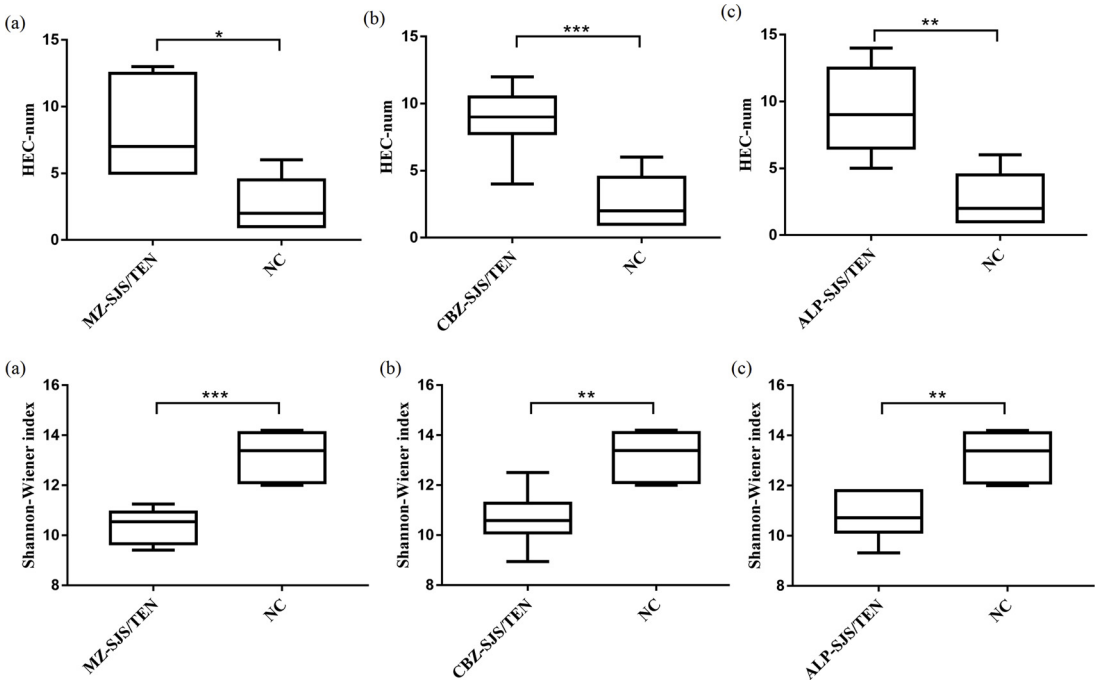

Fig. 2. Comparison of clonal expansion and TCR repertoire diversity in SJS/TEN patients and healthy controls. (A) Comparison of clonal expansion (HEC-number) in SJS/TEN patients and healthy controls. (B) Comparison of TCR repertoire diversity (via Shannon–Wiener index) in SJS/TEN patients and healthy controls.

5. In ALP-induced SJS/TEN patients, there were 2 significantly increased and 51 significantly decreased TRBV/TRBJ combinations ($P < 0.05$, Fig. 5C), with increased combinations including: TRBV7-8/J2-1 and TRBV20-1/J2-1.

4. Discussion

It has been well established that T cells play a critical role in the pathogenesis of SJS/TEN. T-cell activation relies on the TCR to recognize the HLA/antigen complex presented by antigen presenting cells. Therefore, it is important to investigate the composition and variation of the TCR repertoire, which could provide new insights into the underlying disease process. Previous studies assessing T cell repertoires in SJS/TEN have been based on the culture of drug-specific T cells undergoing long-term in vitro culture (Chung et al., 2015; Ko et al., 2011; Yun et al., 2014). However, the in vitro expansion of antigen-specific T cells might distort the T-cell repertoire (Koning et al., 2014). Thus, here we analyse the T cell repertoires directly from

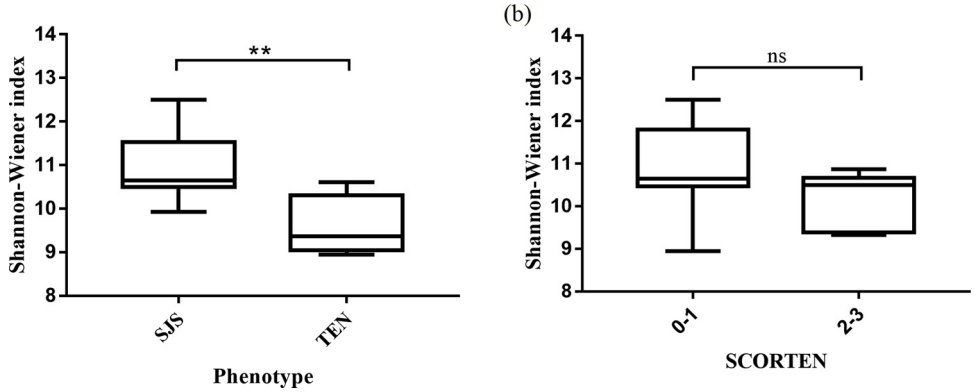

Fig. 3. Relationship between TCR diversity and disease severity. (A) Shannon–Wiener index in patients with different BSA values. (B) Shannon–Wiener index in patients with different SCORTEN values.

peripheral circulating blood. To the best of our knowledge, this is the first study to provide a comprehensive assessment of peripheral blood TCR repertoires in SJS/TEN patients.

First, we assessed the TCRβ repertoire in different samples by analysing the enrichment of expanded clones. The enrichment of expanded clones refers to the number of HECs with a frequency greater than 0.5% of the total clone population, as described in previous studies. We observed that SJS/TEN patients were likely to have more HECs with relatively higher frequencies, while the normal controls had much fewer HECs with relatively lower frequencies. This suggested the presence of oligoclonal proliferation clones in the SJS/TEN patients. Previous studies have demonstrated that the pathogenesis of SJS/TEN involves the activation of T lymphocytes. Activated T cells released various cytokines and chemokines, such as perforin, granzyme B, and granulysin, leading to extensive skin necrosis in SJS/TEN (Su and Chung, 2014). Therefore, our results provide direct evidence that in SJS/TEN patients, T cells not only activate but also expand selectively. Though identical highly expanded clonotypes were not observed among samples of the different SJS/TEN groups, several clonotypes had similar sequences based on phylogenetic relationship analysis in the same drug-induced SJS/TEN groups. These results indicate that oligoclonal proliferation clones in SJS/TEN patients were composed of a set of clones sharing certain specific sequences that interact with the drug and HLA.

Generally, the selective expansion of T cell clones often indicates a restricted TCR repertoire. Thus, we evaluated the diversity of the TCR repertoire in each sample by using H'. H' is commonly used diversity index in ecological research and is now widely used for estimating the diversity of TCR repertoires (Sun et al., 2017; Wu et al., 2015). As anticipated, SJS/TEN patients demonstrated significantly less diversity compared with healthy controls. In other T cell-medicated diseases, it has been reported that altered TCR repertoires are associated with clinical disease severity. For instance, in ankylosing spondylitis, the most severely affected patients had significantly lower TCR repertoire diversity compared with more mildly affected patients (Cui et al., 2018). Therefore, we further analysed the relationship between TCR repertoire diversity and severity of SJS/TEN. First, we found that the TCR repertoire diversity was significantly decreased in TEN patients compared with SJS patients. This suggested that low TCR repertoire diversity correlates with high degree of skin necrosis. SCORTEN, a score system that was developed from a logistic regression model in France, is emerging as a standard for assessing SJS/TEN severity (Roujeau and Bastuji-Garin, 2011; Sekula et al., 2011). It can be easily and quickly calculated on the basis of seven parameters within the first 24 h after admission. We previously reported that SCORTEN was also applicable to Chinese patients with SJS/TEN and could offer accurate information about prognosis (Zhu et al., 2012). Patients with high SCORTEN values are considered to have a poorer prognosis. In this study, we found that the TCR repertoire diversity in patients with scores of 2–3 by SCORTEN was slightly lower than in patients with a SCORTEN score of 0–1, though it did not achieve statistical significance. This result indicated that the low TCR repertoire diversity might associate with poor prognosis in SJS/TEN patients and more cases are needed to validate the

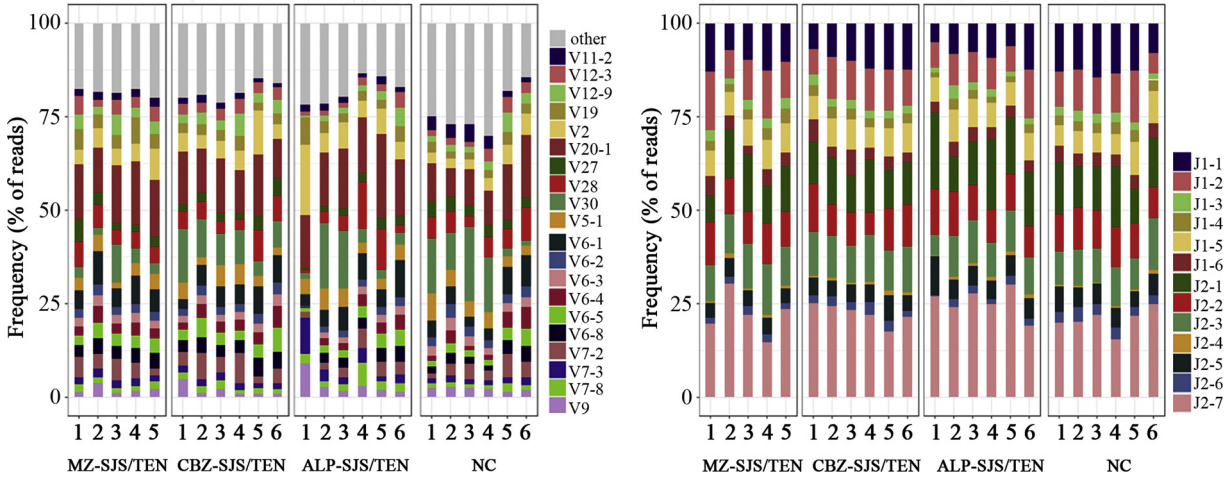

Fig. 4. The top 20 most-expressed genes of the V gene and J gene segments from each sample. The X-axis depicts each subject; the Y-axis corresponds to the percentage of each TRBV and TRBJ gene type. (A) V genes of each sample. The top 20 TRBV genes are indicated in various colours, and the remaining gene types are grouped together in black. (B) J genes of each sample. All the J genes are indicated in various colours (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

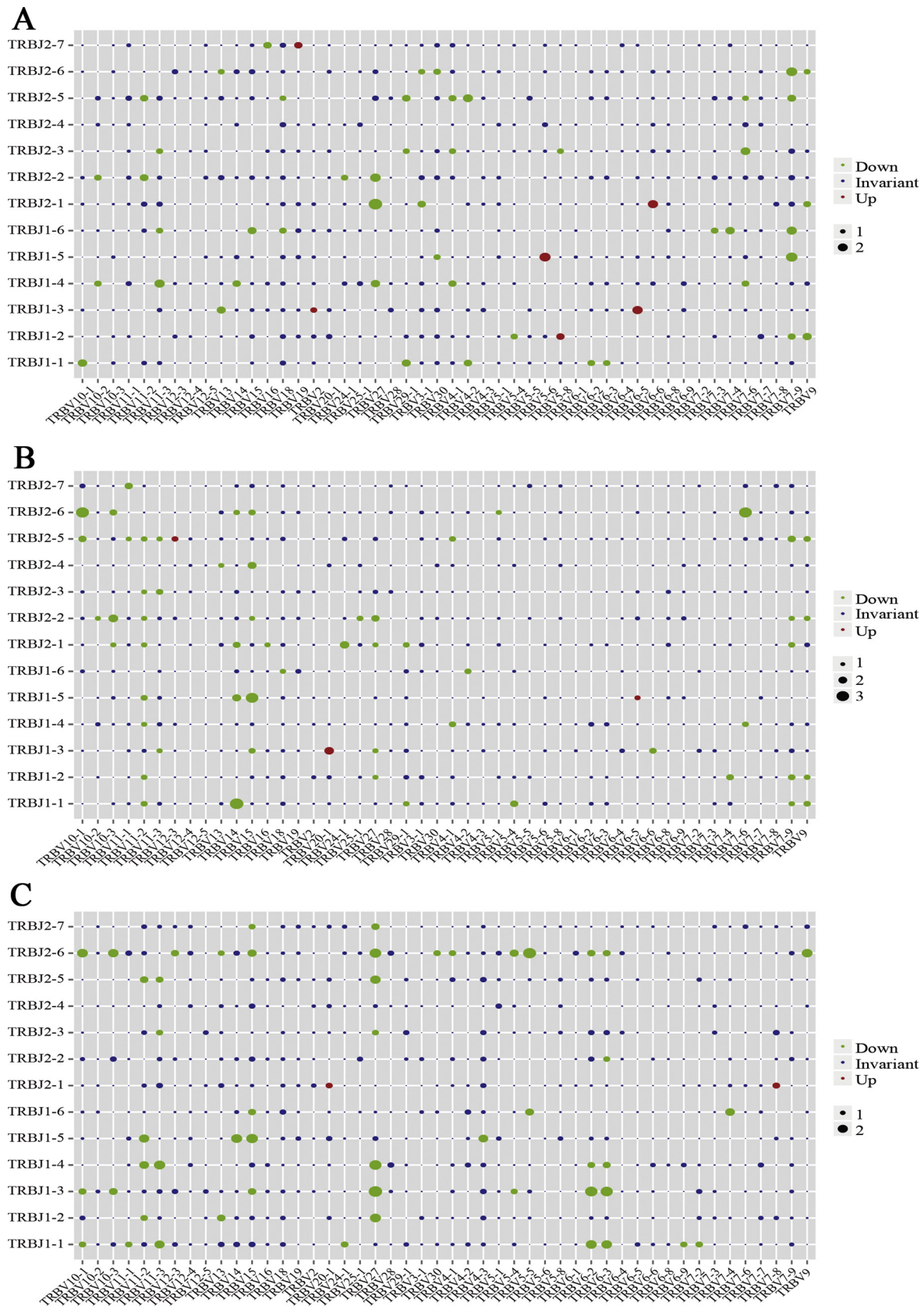

Fig. 5. Comparison of TRBV/TRBJ combination frequencies in SJS/TEN patients and healthy controls. The X-axis indicates the TRBV subtype, and the Y-axis indicates the TRBJ gene subtype. Blue dots represent TRBV/TRBJ combinations where no significant difference was observed between each group. Red dots represent TRBV/TRBJ combinations that were upregulated significantly. Green dots represent TRBV/TRBJ combinations that were upregulated significantly. The size of each bubble corresponds to the level of significance. (A) MZ-SJS/TEN group vs NC group, (B) CBZ-SJS/TEN group vs NC group, and (C) ALP-SJS/TEN group vs NC group (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

results.

Abnormalities in diversity and clonality could result from deviations in V and J gene usage. Therefore, we analysed the usage level of V/J subtypes and VJ combination in each group. Different usages of V/J subtypes and VJ combination were found in different drug-induced SJS/TEN patients compared with normal controls. Abnormal TRBV/TRBJ usage and VJ combination might be associated with the immunological pathogenesis of the disease. In a study of TCR repertoires in systemic lupus erythematosus patients using high-throughput sequencing, the expression levels of 10 TRBV segments, 6 TRBJ segments and 73 V J combinations were significantly different in the systemic lupus erythematosus group (Sui et al., 2015).

There were certain limitations in the present study. First, because of the low morbidity of SJS/TEN and our strict inclusion criteria, the sample size was not large. Second, due to the lack of analysis from skin samples, our study was restricted from examining the changes in TCR repertoires of the T cells infiltrating the skin. However, previous studies in other skin diseases have shown that skewing of the TCR repertoire could be detected in both skin tissues and peripheral blood of affected patients, and expanded TCR clones were shared between these two different samples (Brooklyn et al., 2007; Gotoh et al., 2008). Since previous studies have shown that peripheral blood cells have the ability to respond differentially to varying perturbations occurring anywhere in the body, the alterations of TCR repertoires and the oligoclonal T cells of the blood could partially reflect alterations of the T cells infiltrating in the skin. Third, due to the limitations of collecting sufficient fresh peripheral blood, we did not sort the T cells into different subsets for further analysis. Histopathology has previously showed that the infiltrating T cells in the skin or blister fluid of SJS/TEN patients were mostly CD8 + T cells (Hoetzenecker et al., 2016). However, in vitro cultures, the drug-expanded T cells from SJS/TEN patients were composed of both CD4+ and CD8 + T cells (Chung et al., 2015; Wu et al., 2007). Thus, the exact function of each T cell subset in the pathogenesis of SJS/TEN remains to be further examined.

In conclusion, our study provided detailed characteristics of peripheral blood TCR-β repertoires in SJS/TEN patients through high-throughput sequencing. We found that SJS/TEN patients had more highly expanded clones and less TCR repertoire diversity, and the TCR repertoire diversity of patients might be associated with the clinical severity of disease. We also observed several different-usage TRBV/TRBJ subtypes and combinations, which provides more detailed insights into the TCR-β repertoires of SJS/TEN patients. Future investigations should recruit more cases, collect skin samples and sort T cell in to different subsets for specific analysis.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2019.01.002.

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