The study of severe cutaneous drug hypersensitivity reactions from a systems biology perspective

James R. Perkins[a], *Pedro Ayuso*[a], *José A. Cornejo-García*[a,b], *and Juan A. Ranea*[c,d]

Purpose of review
Stevens–Johnson syndrome and toxic epidermal necrolysis are severe hypersensitivity reactions, the majority of which are drug induced. The underlying mechanisms are not fully understood. Here, we review recent findings concerning both mechanistic and genetic factors related to these diseases and propose future approaches to unravel their complexity.

Recent findings
Genome-wide association study studies have identified several variants in the human leukocyte antigen region associated with these reactions. These are highly dependent on the population studied and the triggering drug. The T-cell receptor repertoire of the patient is also key. Fas–Fas ligand interactions, perforin and granulysin have also been identified as important players. Furthermore, a high-throughput gene expression study has identified a number of genes that increase in expression in patients during the acute phase of these reactions.

Summary
We review recent high-throughput studies on these diseases and suggest ways in which the data can be combined and reanalyzed using integrative systems biology techniques. We also suggest future lines of research using recent technology that could shed further light on their underlying mechanisms.

Keywords
human leukocyte antigen, hypersensitivity drug reactions, Stevens–Johnson syndrome, systems biology, T cells, toxic epidermal necrolysis

INTRODUCTION

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are two related, life-threatening conditions, induced by drugs in the majority of cases [1]. They are characterized by target-like lesions and blisters on erythematous macules occurring all over the body, particularly the trunk, in addition to mucosal involvement and skin detachment [2,3]. They can be considered part of a spectrum of severe cutaneous reactions, differentiated according to the extent of skin detachment (<10%: SJS; 10–30%: SJS–TEN overlapping; >30%: TEN) [2] and we will sometimes refer to them as a single disease. Mortality is high, ranging from 10% (SJS) to 50% (TEN). However, prevalence is low with five to six cases per million patients for SJS, and one to two cases for TEN [4,5]. The medicines commonly implicated include aromatic antiepileptic drugs [carbamazepine (CBZ), phenytoin and phenobarbital], the antihyperuricemic allopurinol, the reverse-transcriptase inhibitor abacavir, anti-infective sulfonamides (especially cotrimoxazole) and oxicam-NSAIDs [1,6,7]. Work in recent years has revealed a strong genetic component, including association with specific human leukocyte antigen (HLA) alleles. However, the precise genotype varies greatly depending on the drug and

[a]Research Laboratory, IBIMA, Regional University Hospital of Malaga, UMA, [b]Allergy Unit, IBIMA, Regional University Hospital of Malaga, UMA, [c]Department of Molecular Biology and Biochemistry, Faculty of Sciences, University of Malaga and [d]CIBER de Enfermedades raras (CIBERER), Malaga, Spain

Correspondence to Dr José Antonio Cornejo-García, Laboratorio de Investigación, Plaza del Hospital Civil s/n, Hospital Civil, Pabellón 6, sótano. 29009 Malaga, Spain. Tel: +34 952 290 346; fax: +34 952 290 302; e-mail: josea.cornejo@ibima.eu

Curr Opin Allergy Clin Immunol 2014, 14:301–306
DOI:10.1097/ACI.0000000000000076

KEY POINTS

- Few high-throughput omics data sets exist that profile SJS and TEN at the molecular level.
- Study has been made into the involvement of specific HLA alleles, at the genetic and functional level; however, the exact mechanisms are not well understood.
- The generation of new data sets and their integration using systems biology techniques will be key to a complete understanding of these diseases.
- Excellent progress has been made applying systems biology to other fields including allergy and asthma.

the population studied [1,5]. The aetiopathology is thought to be highly complex and is still not fully understood. While certain HLA alleles increase the risk of reactions, the majority of individuals with the risk allele will not react, pointing to the involvement of other factors. Some of these are known, such as the T-cell receptor (TCR) repertoire [8,9]; however, there may be other genetic and environmental cofactors.

This interplay between different drugs, population-specific genetic factors and the environment makes the elucidation of the mechanisms underlying SJS and TEN difficult. The high-mortality rate and lack of a generalizable genetic test for these conditions make it a problem that requires our immediate attention [4,5].

In this review, we propose methods for the study of severe cutaneous drug hypersensitivity reactions, using SJS and TEN as a model, applying systems biology. We suggest ways to integrate the results of published studies with other data sources, including protein interactions and nonexonic genome annotation. We also suggest future high-throughput experiments and analyses that could help shed light on this disease.

UNDERLYING MECHANISMS AND GENETIC FACTORS

Different mediators produced by cytotoxic T lymphocytes (CTL) and natural killer (NK) cells are thought to be key players in these immune reactions, as they are the main cell types present in blisters from skin lesions in SJS/TEN [10]. Fas–Fas ligand (FasL) interaction is considered to be one of the most important mechanisms in keratinocyte apoptosis through caspase pathway activation [11–13]. However, it has also been reported that anti-Fas monoclonal antibodies do not attenuate the cytotoxic effects on keratinocytes, which diminish when perforin/granzyme B expression is inhibited [14]. Increased levels of perforin, granzyme B, tumor necrosis factor (TNF)-α and FasL have been shown to correlate with severity in allergic reactions to drugs, from mild rashes to TEN [15]. Another interesting cytotoxic protein that has also recently been reported to play a key role in widespread keratinocyte apoptosis in SJS/TEN is granulysin [10,16]. The complexity of the mechanisms underlying these diseases is also highlighted by the participation of innate immune molecules such as alarmins [17,18]. Apart from TNF-α, a number of other cytokines and chemokines participating in T-cell activation, proliferation or trafficking have been implicated in SJS/TEN. These include interleukin (IL)-2, IL-5, IL-6, IL-10, IL-13, interferon (IFN)-γ, CCR3, CXCR4 and CCR10-CCL27 [19–22].

The association of HLA alleles with SJS/TEN was first reported by Roujeau *et al.* [23] more than 25 years ago. Since then, specific HLA alleles have been found to be associated with this disease depending on the population studied. For instance, in a study by Chung *et al.* [24], the HLA-B*1502 allele was present in all Han-Chinese patients suffering from CBZ-induced SJS, but only in 3% of CBZ-tolerant individuals and 9% of healthy controls, with similar results in a second follow-up study [25]. The association of this allele with CBZ-induced SJS/TEN has been found in various other studies, which have recently been reviewed [26▪]. In fact, the US Food and Drug Administration (FDA) advises genetic screening prior to the use of different drugs for certain populations. However, the participation of this allele in SJS/TEN is far from universal, and an association was not found in studies of Japanese [27] or European populations [28], probably because of the low prevalence of this allele. Most genetic studies of SJS/TEN induced by allopurinol have found associations with the HLA-B*5801 allele in both Asian [29–31] and European populations [32]. A recent genome-wide association study (GWAS) found a number of polymorphisms showing association with allopurinol-induced SJS/TEN localized on chromosome 6. The strongest signal was for rs2734583 in *BAT1*, which belongs to a cluster of genes in close proximity to those encoding TNF-α and TNF-β. The SNP rs3094011 in *HCP5* also showed strong association [33]; this variant is also associated with SJS/TEN in patients treated with abacavir [34]. Interestingly, a SNP in psoriasis susceptibility 1 candidate 1 (*PSORS1C1*) gene, which is in absolute linkage equilibrium with HLA-B*5801 [35], also showed statistically significant association with these reactions [33].

Although significant advances have been achieved in our understanding of the pharmacogenomics of SJS/TEN over the last decade, the

mechanisms responsible for the interactions between drugs, the HLA system and T cells are complex and still not understood. The hapten model proposes that protein modifications by the binding of drugs (or drug metabolites) leads to the formation of new, more antigenic determinants that elicit an immunological response [36]. Another possibility considers that drugs can also reversibly bind to TCRs or to HLA–peptide complexes or both and trigger a reaction (pharmacological interaction with immune receptor or pi-model) [37]. It has also been postulated that only drugs that can induce particular types of cell damage are able to induce an immune-mediated drug reaction (danger model) [38]. This is backed up by the finding of an increased expression of several endogenous damage-associated molecular patterns (alarmins) in severe bullous diseases induced by drugs [17,18]. However, none of the previous models can explain the complexity of these reactions alone. In 2008, Chessman *et al.* [39] elegantly demonstrated that CD8 lymphocyte activation required the specific binding of abacavir with the HLA-B*5701 allele and that the affinity of antigen binding laid in the tridimensional structure of the peptide-binding pocket. Conformational changes induced by drugs can alter the binding between HLA and endogenous proteins, generating new self-immunological peptides inducing a hypersensitivity drug reaction [40,41].

Interest exists in the potential predictive value of genetic variants associated with SJS/TEN and other severe cutaneous reactions induced by drugs before starting a pharmacological treatment. Abacavir-induced hypersensitivity reactions are one example of the successful translation of pharmacogenetics to personalized medicine. Various prospective studies [42–45] conducted on different populations have shown the benefits of HLA-B*5701 screening in order to reduce the incidence of these reactions.

However, our current knowledge from pharmacogenetic and mechanistic studies cannot fully explain SJS/TEN-associated risks as other factors may also have a role, including different proteins/enzymes related to drug metabolism, T-cell receptors, individual predisposing factors and gene–gene and gene–environment interactions. The integrative approaches and new technologies that will be discussed here could be used to improve our understanding of this disease and the clinical management of patients.

HIGH-THROUGHPUT DATA SETS

The past few years have seen a large number of GWAS generated for this disease in European [46–48] and Japanese populations [33,49,50]. However, few studies have been made of the reaction itself at the functional genomic level, perhaps due in part to the low prevalence of the disease and difficulty of obtaining samples in the acute phase. Currently, a single high-throughput gene expression study [17] exists, from our group, comparing gene expression in peripheral blood mononuclear cells from patients with various severe cutaneous drug-induced hypersensitivity reactions, including SJS and TEN. This is in contrast to more common drug hypersensitivity reactions such as NSAID hypersensitivity, where gene expression and methylation have been measured for different tissues using microarrays [51–53]. Given the gaps in our current knowledge of the pathomechanisms involved, more functional genomic and proteomic experiments will be key to elucidating the regulatory changes that occur and to find potential new biomarkers by suggesting new targets for future genetics-based analyses. In the next section, we will suggest further experiments that could be undertaken as well as how we can integrate their results.

SYSTEMS BIOLOGY ANALYSES

Rather than offer our own definition of systems biology, we will use the interpretation provided by Arazi *et al.* [54]. They loosely divide the subject into two: 'data-driven' and 'hypothesis based'. The former consists of the generation of high-throughput data sets that assay the genome in a systematic, unbiased way, for example, using microarrays or next-generation sequencing. These data must then be integrated, analysed and interpreted in an appropriate way. Hypothesis-based systems biology approaches view the problem from a different angle. An *a priori* selection is made of what will be investigated, for example, a pathway that is known or expected to be involved in a given disease. Data are then generated for this pathway, and a model is then fitted that explains the data. The aim is then to test how good the model is by using it to make predictions (i.e. by perturbing components of the model) and comparing the results to real-world data [54]. For SJS and TEN, there are a number of data sets that are appropriate for a data-driven analysis; however to the best of our knowledge, no study has been made using hypothesis-based model driven approaches.

META-ANALYSIS AND DATA INTEGRATION

As mentioned above, most high-throughput studies [33,46–50] of SJS and TEN have been GWAS. Recently, meta-analyses have been performed to look at the influence of HLA-B alleles on carbamazepine [26▪] and allopurinol induced reactions [55].

Although almost all GWAS found no significant associations outside of the HLA region of chromosome 6, but by combining studies and thus increasing power, variants may be found in other genomic regions. These variants may be necessary along with specific HLA alleles or other factors to trigger reactions. In order to further clarify the function of such variants, additional data sources must be used. The variants should be mapped to genes and can be combined with expression data in order to probe the effects of the variants on gene expression. The analysis can be extended to include relationships, such as protein interactions, comembership of metabolic or signalling pathways, coexpression data or evolutionary co-occurrence [56,57]. The data could be used to construct a network that would allow us to visualize potential relationships between SJS/TEN associated genes and provide clues as to the processes underpinning the reactions. Such an approach has been used by Renkonen *et al.* [58,59] to investigate genetic variation relating to asthma and acute allergic diseases.

Another approach to investigate the SNPs associated with SJS/TEN is to focus on the SNPs falling outside of coding regions. These variants account for the majority of disease-associated SNPs [60]; however, determining the functional role of this variation can be challenging [61▪▪]. Potential approaches include in-silico annotation of the genome, using data from projects such as ENCODE [62], which aims to provide annotation for non-exonic regions of the genome including areas of open chromatin, DNA–protein interaction, histone modifications and RNA expression. Investigation of noncoding variation has led to the discovery of a regulatory sequence in the 3′ UTR of HLA-C that affects the binding of microRNA miRNA-148, changing expression levels of HLA-C [63,64]; given the strong association of SJS/TEN with HLA-B experiments similar approaches to investigate noncoding RNA involvement in regulation could prove fruitful.

Combining high-throughput genotypic and expression data provides a way to find genomic regions that affect gene expression by searching for expression quantitative trait loci [65]. A similar approach has been undertaken by Couto Alves *et al.* [66▪▪] to investigate the mechanisms underlying a different class of allergic reaction. They combined microarray derived CD4+ T-cell expression data in allergic rhinitis patients with the results of a GWAS study for individuals allergic to grass-pollen. By using gene-enrichment and network construction techniques, they detected the involvement of complement system-related pathways in both data sets and identified genes that linked the complement system and T-cell activation [66▪▪].

FUTURE EXPERIMENTAL DESIGNS USING HIGH-THROUGHPUT TECHNOLOGY AND SYSTEMS BIOLOGY

Future experiments should use RNA-seq over microarrays to measure expression because it has been shown to outperform them in several ways [67] and allows the detection of novel transcripts and alternative splicing [68], potentially allowing us to determine the effects of genetic variation on differential exon usage and the expression of noncoding RNAs [61▪▪]. This could be combined with further data sets such as transcription factor binding patterns determined through the use of ChIP-seq to investigate mutations in transcription factor binding regions and their effect on gene expression [69].

Recent technologies have allowed us to investigate the TCR repertoire in unprecedented detail. In this approach, next-generation sequencing technology is used to analyze the complementary determining region 3 of the TCR beta chain. This field is progressing rapidly and has been applied to leukaemia [70,71] and to profile TCR diversity [72,73]. It is expected to be of utility in the study of SJS/TEN given the importance of the TCR repertoire in these reactions [9] and could be used to compare TCR diversity between case and control participants, or used to look for correlations with disease severity and different culprit drugs.

Polypharmacology offers another potential avenue of investigation that should be explored in the context of SJS and TEN. The classical, silver bullet paradigm of drug design, where drugs were seen as selective ligands acting on a single target, has long been disputed. It is being succeeded by the more complex view that many drugs can act on the same target while conversely the same drug can bind multiple targets [74▪,75,76]. This has implications for the study of SJS/TEN, and the possible off target binding of the drug under investigation should be taken into account when searching for the antigenic determinants responsible for the elicitation of a reaction. Important resources in this area include DrugBank, containing detailed information on thousands of FDA approved and experimental drugs as well as their targets [77]; ChEMBL, containing details of the chemical properties of various bioactive small molecules [78]; and STITCH, containing data for known and predicted interactions between chemicals and proteins for multiple species [79].

CONCLUSION

There are now several high-throughput data sets investigating the puzzle of SJS and TEN, most of which are focussed on genetics. Most findings have been related to the HLA genomic region. However,

further research is needed in order to investigate the dynamics of the interactions between specific HLA alleles, drug metabolites and T cells. In this review, we have suggested potential ways forward for the analysis of current high-throughput data sets and presented potential future steps to further elucidate the molecular bases of these diseases. However, obtaining acute-phase samples remains a key bottleneck in the study of these diseases, because of the lack of adequate animal models alongside their relatively low prevalence. Future experiments must therefore be well designed to optimize the use of these samples. It is clear that future breakthroughs will require close collaboration between researchers from a variety of fields including immunology, molecular biology, pharmacology, genetics and bioinformatics.

Acknowledgements

This work has been supported by grants from Carlos III National Health Institute RD12/0013/0001 (RIRAAF network), FIS PI12/02247 and CIBER de Enfermedades Raras; Marie Curie (IAPP 7th Framework Program Mr. SymBioMath, no. 324554); European Commission: Systems Microscopy consortium (FP7/2007-2013-258068); Spanish Ministry of Economy and Competitiveness (SAF2012-33110); Andalusian Government and FEDER (CTS-486); and The Health Government of Andalusia (PI-0279-2013).

Conflicts of interest

There are no conflicts of interest.

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