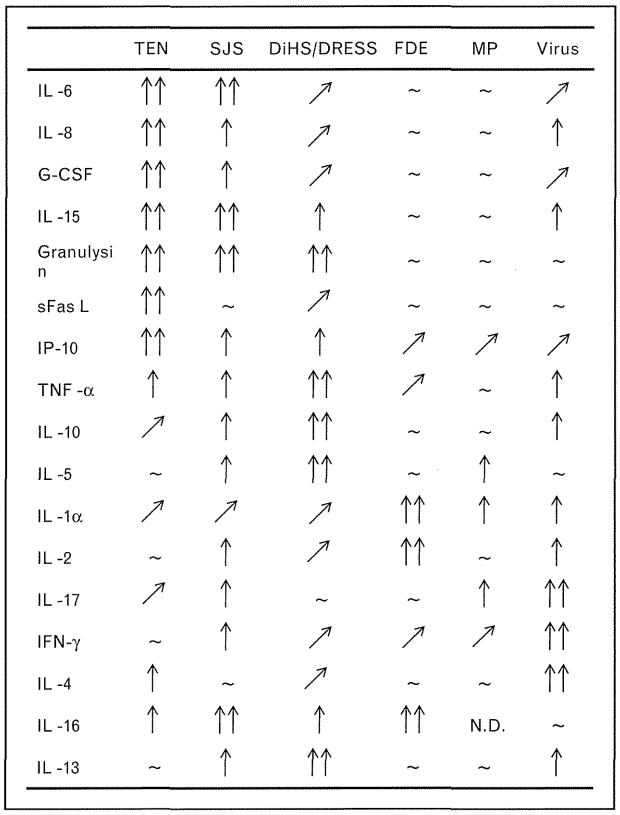

	TEN	SJS	DiHS/DRESS	FDE	MP	Virus
IL-6	↑↑	↑↑	↗	~	~	↗
IL-8	↑↑	↑	↗	~	~	↑
G-CSF	↑↑	↑	↗	~	~	↗
IL-15	↑↑	↑↑	↑	~	~	↑
Granulysin	↑↑	↑↑	↑↑	~	~	~
sFas L	↑↑	~	↗	~	~	~
IP-10	↑↑	↑	↑	↗	↗	↗
TNF-α	↑	↑	↑↑	↗	~	↑
IL-10	↗	↑	↑↑	~	~	↑
IL-5	~	↑	↑↑	~	↑	~
IL-1α	↗	↗	↗	↑↑	↑	↑
IL-2	~	↑	↗	↑↑	~	↑
IL-17	↗	↑	~	~	↑	↑↑
IFN-γ	~	↑	↗	↗	↗	↑↑
IL-4	↑	~	↗	~	~	↑↑
IL-16	↑	↑↑	↑	↑↑	N.D.	~
IL-13	~	↑	↑↑	~	~	↑

FIGURE 1. Increased cytokine/chemokines in sera of patients with adverse drug reactions and viral exanthem at their initial presentation before typical clinical symptoms develop. Each symbol represents the magnitude of alterations of cytokine/chemokine levels in patients when compared with the mean cytokine/chemokine levels in healthy controls. Double 'increase' or 'decrease' symbol indicates a profound increase or decrease. Single 'increase' or 'decrease' symbol indicates an increase or decrease. Wavy lines represent no significant alterations. Slanting 'increase' represents a slight increase. N.D., not done.

apparently preceded the appearance of the full-blown pictures by days, these cytokines could serve as both a diagnostic and a predictive tool in monitoring patients with ADRs. These findings suggest that both IL-6 and IP-10 might be involved in the early event required for the development of severe ADRs but may not be unique to each clinical phenotype as late events; although SJS/TEN and DiHS/DRESS represent opposite ends of severe ADRs, they may share important common pathophysiologic processes. Although additional studies are needed to determine whether IL-6 and IP-10 act synergistically or independently, serum IL-6 and IP-10 levels at the initial presentation could be promising surrogate biomarkers for predicting disease progression to severe ADRs. Similar tendency was also observed in the IL-8 and granulocyte-colony stimulating factor (G-CSF) levels: increased levels of IL-6, IL-8, G-CSF and IP-10 may reflect the severity of the disease, indicating an injurious outcome.

Importantly, serum sFas L levels at the initial presentation were the highest in TEN patients who subsequently progressed to TEN, but not to SJS. Although sFas L [18–22] and granulysin [23,24] have been implicated in the pathological process of SJS/TEN, these findings suggest that sFas L represents a useful early biomarker that can predict the subsequent progression to TEN, but not SJS, particularly when combined with the increase in serum IL-6 and IP-10 levels (Fig. 2). Because TEN has been placed in the same disease category with SJS, we were surprised to find a highly significant difference in the sFas L level between SJS and TEN at the earliest stage. Thus, serum sFas L levels at the initial presentation can be used for effectively distinguishing patients at the risk of subsequently developing TEN from patients with SJS.

Surprisingly, the IL-17 level was significantly increased in patients with viral exanthema but not in these severe ADRs and rather decreased in DiHS/DERESS patients, although there was no statistically significant difference among these ADRs groups. Although IL-17 has been implicated in the pathogenesis of inflammatory and autoimmune diseases [25,26], we did not detect a correlation with the serum level and the disease phenotype of ADRs. This was an unexpected finding because it has been reported that IL-17 expression was increased in TEN and its level correlated with clinical severity of the disease [27]. This could be explained by the fact that the first measurement of cytokine levels in our cohort was performed at the earliest time point, 3–7 days after onset, whereas previous studies measured samples that were taken at later time points (usually 2 weeks after onset), even after starting therapy. In view of our finding that the IL-17 levels rather increased after starting treatment with corticosteroids, increased IL-17 levels that had been previously reported in TEN may have resulted from a possible delay in taking serum samples during the disease course. Alternatively, it is tempting to speculate that increased serum IL-17 levels during the course of the disease may serve to prevent progression to severe ADRs and may be bifunctional depending on other cytokine levels.

Unlike other Th1-type cytokines, the IL-2 level appeared to be the opposite of what would be expected if increased production were simply a Th1-type response: the IL-2 level was the highest in FDE, a milder form of ADRs, whereas the increase was not observed in other ADRs. If this finding can be validated, high IL-2 levels could be interpreted as increased IL-2 levels being early biomarkers

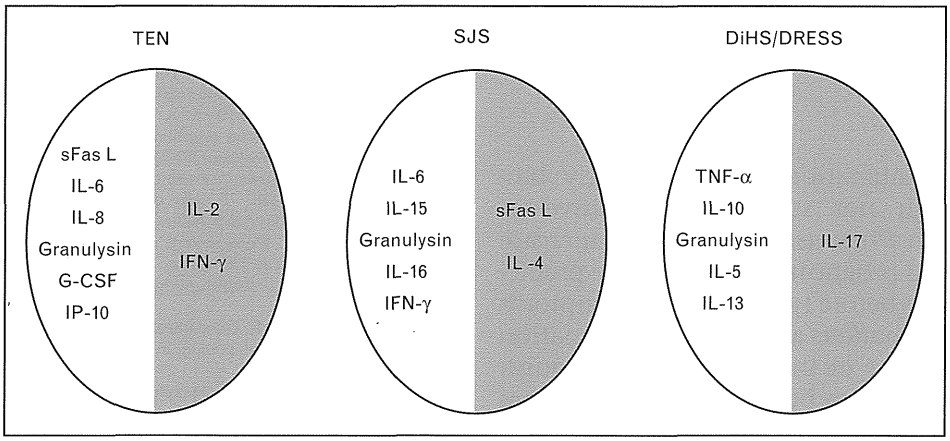

FIGURE 2. Combinations of multiple early biomarkers useful for predicting progression to severe ADRs. A light area indicates increased cytokines/chemokines in each type of severe ADRs. A dark area indicates decreased cytokines/chemokines. ADRs, adverse drug reactions.

predictive of beneficial outcomes: the IL-2 levels, particularly when combined with increased IL-16 levels, may predict better prognosis. To determine the physiologically relevant source of IL-16, we have recently analyzed skin lesions of severe ADRs, SJS/TEN, by using immunohistochemistry with anti-IL-16 and antitryptase antibodies. The results show that expression of IL-16 was colocalized with tryptase-positive mast cells (Mizukawa Y *et al.* article submitted). Because accumulation of IL-16-positive mast cells beneath the epidermis was preferentially observed in the vicinity of Foxp3$^+$ regulatory T cells (Tregs) in FDE lesions, but not in SJS/TEN lesions, a likely interpretation of these observations, in consideration of the data presented here, is that mast cells residing in the FDE lesions represent a major source of IL-16 in the setting of ADRs and that mast cells contribute to the resolution of ADR lesions by recruiting Tregs through the release of IL-16. This emphasizes the importance of Tregs as a negative regulator of TEN [28▪▪,29▪,30]. Interestingly, the IL-16 levels were significantly increased not only in FDE and SJS, but also in DiHS/DRESS patients, but not in TEN patients.

Contrary to our initial expectation, increased IL-10 levels were observed in SJS and DiHS/DRESS, but not in TEN. Because IL-10 generally acts as an immunosupression during inflammatory diseases [11,12], the serum IL-10 level was initially thought to be associated with milder forms of ADRs. However, the increase was associated with the development of severe ADRs. Nevertheless, one surprising finding was that serum IL-10 levels in SJS were much higher than those in TEN despite clinical similarities between the two diseases. Our data could be interpreted as an indication that increased IL-10 levels associated with increased levels of IL-2, IL-5 and IFN-γ at the initial presentation may be a useful predictor of SJS patients who will not progress to TEN: alternatively, relatively low levels of IL-10 associated with increased IL-6 and IP-10 levels could be interpreted as suggesting the subsequent progression to TEN.

As systemic corticosteroids are recommended for the treatment of many ADRs, and could have a significant impact on the levels of these biomarkers' levels, it is important to determine to what extent these markers could be influenced by the systemic corticosteroids and the treatment outcomes. However, although our preliminary findings indicate that many of these biomarkers were profoundly decreased in ADR patients after starting systemic corticosteroids, it would be premature to conclude which biomarkers could be prospectively used for predicting patients' clinical response to systemic corticosteroids before starting treatment with oral prednisolone. Despite it being well recognized that skin biopsies are also of value for diagnosing severe ADRs at the earliest stage of the disease, measurements of these biomarkers, rather than biopsy samples, are more practical, noninvasive and easily repeatable approaches for identifying patients at risk of progressing to severe ADRs and those who may respond to treatment. This is because a biopsy sample only represents a 'snapshot' during the dynamic process and time course of the ADR as it progresses. An important limitation of the present study was the relatively small number of samples from patients with severe ADRs that were available for our analysis, especially, because samples from patients who had received systemic corticosteroids before their initial presentation were excluded from this study and those from patients who had been complicated by apparent bacterial and viral infections at the initial presentation were also excluded. Thus, this limitation needs to be

taken into account when drawing conclusions from the data presented.

CONCLUSION

The use of a combination of several early biomarkers, although not sufficiently sensitive or specific on its own when used alone, could increase the diagnostic and prognostic utility for the prediction of severe ADRs before typical clinical symptoms develop. These biomarkers also provide information on the clinical course of the disease to distinguish patients destined to have beneficial outcomes from those who will have poor outcomes. Identifying patients with a high risk of subsequently progressing to severe ADRs by such a noninvasive method is relevant for clinical decision-making, as these patients may benefit from timely and selective therapeutic interventions. These biomarkers may represent novel therapeutic targets that could be inhibited by future anticytokine treatment. The findings presented here provide the theoretical basis for the use of various biologicals for control of the evolution of severe ADRs, for which no specific therapy has received wide acceptance as the best evidence-based therapy in patients with severe ADRs to date. This search should be more widely validated to assess the exact sensitivity and specificity of these measurements in a larger, independent, prospective, multicenter study. This should include samples from patients with other inflammatory diseases other than ADRs, because of the rarity of severe ADRs.

Acknowledgements

None.

Financial support and sponsorship

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (to T.S.) and the Health and Labour Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan (to T.S.).

Conflicts of interest

There are no conflicts of interest.

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Acta Derm Venereol 2016; 96: 111–113

SHORT COMMUNICATION

Synergistic Effects of *Mycoplasma pneumoniae* Infection and Drug Reaction on the Development of Atypical Stevens-Johnson Syndrome in Adults

Maiko Kurata, Yoko Kano*, Yohei Sato, Kazuhisa Hirahara and Tetsuo Shiohara
Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka, Tokyo 181-8611, Japan. E-mail: kano@ks.kyorin-u.ac.jp
Accepted Jun 15, 2015; Epub ahead of print Jun 18, 2015

Mycoplasma pneumoniae (Mp) is one of the most common causes of community-acquired pneumonia. It can induce a cellular immune response, leading to respiratory inflammation and injury. Mp infection is frequently accompanied by a variety of extrapulmonary manifestations, including arthritis, hepatitis, myositis, neurological involvement and cutaneous diseases. Cutaneous diseases, such as erythema nodosum, erythema multiforme, urticaria, vasculitis and Stevens-Johnson syndrome (SJS), develop in up to 25–33% of all Mp infections (1, 2). SJS associated with Mp infection is commonly observed in children, while adult SJS is caused mainly by drugs (3). Adult SJS associated with Mp infection has seldom been reported (4, 5). Here, we report 2 adult SJS patients with Mp infection and drug reaction, with possible synergistic effects on the development of SJS.

CASE REPORTS

Patient 1. A 33-year-old man visited our hospital with ocular and oral lesions. He had a high-grade fever, general fatigue and nasal discharge, for which he had been prescribed diclofenac and L-carbocisteine by his doctor one day after the appearance of symptoms. On the following day, he had a painful throat and eyes, and was admitted to our hospital. Physical examination revealed hyperaemic conjunctivae, corneal erosions and pseudomembranous formation. Erosions on the buccal mucosa and ulcerations on the lips were also observed. No cutaneous lesions were seen. Laboratory tests revealed leukocytes 13.3×10^9/l and C-reactive protein (CRP) 7.1 mg/dl (normal <0.3). Liver function tests were within normal limits. Human immunodeficiency virus (HIV) infection was negative and no adenovirus antigen was detected in the conjunctivae. Herpes simplex virus (HSV) antigen on the lip was negative. On admission, a titre of particle agglutination (PA) test for Mp was 1:320 (normal <40). No abnormal findings were seen on chest X-ray. A skin specimen could not be obtained because the patient declined biopsy of the labial lesions. Atypical SJS without appearance of skin lesions was suspected. Lymphocyte transformation test (LTT) was performed to identify culprit drugs on admission (6, 7). The LTT for diclofenac was positive (stimulation index level 2.56 (positive >1.80)). The patient was treated with oral prednisolone, 40 mg daily, and a glucocorticoid eye drop. The prednisolone was tapered gradually. A 4-fold reduction in PA titre was found 4 weeks after onset.

Patient 2. A 59-year-old man was referred to our hospital because of a high-grade fever and mucosal lesions. He had been treated with loxoprofen and clarithromycin for fever and sore throat for 4 days before presentation. The symptoms persisted and he noticed labial and oral lesions. On examination, his temperature was 40°C, and bilateral conjunctivitis with pseudomembranous formation, corneal erosions (Fig. 1A), haemorrhagic appearance of the lips, and erosions on the buccal mucosa (Fig. 1B) were observed. Erosions were seen on the glans penis. Several scattered macules with bullae were also observed on the trunk and upper extremities (Fig. 1C). Laboratory findings on admission showed leukocytes 6.3×10^9/l and a CRP level of 18.0 mg/dl. Mild liver dysfunction was detected. A PA titre for Mp was 1:20,480 after admission, and then significantly decreased. Adenovirus antigens in the conjunctivae and HSV antigen on the lip were negative. Chest X-ray showed mild infiltrative shadowing on the right lower lung field. The LTT for loxoprofen was positive (stimulation index level 4.57) (6, 7). Histological examination of a biopsy specimen of an erythematous macule on the abdomen revealed epidermal necrosis and a mild lymphocytic infiltration in the upper dermis (Fig. 1D). A diagnosis of atypical SJS was made, and treatment with oral prednisolone, 60 mg daily, and a glucocorticoid eye drop were initiated. The erythematous rash resolved, and then the oral lesions steadily improved. The infiltrative shadow on the chest

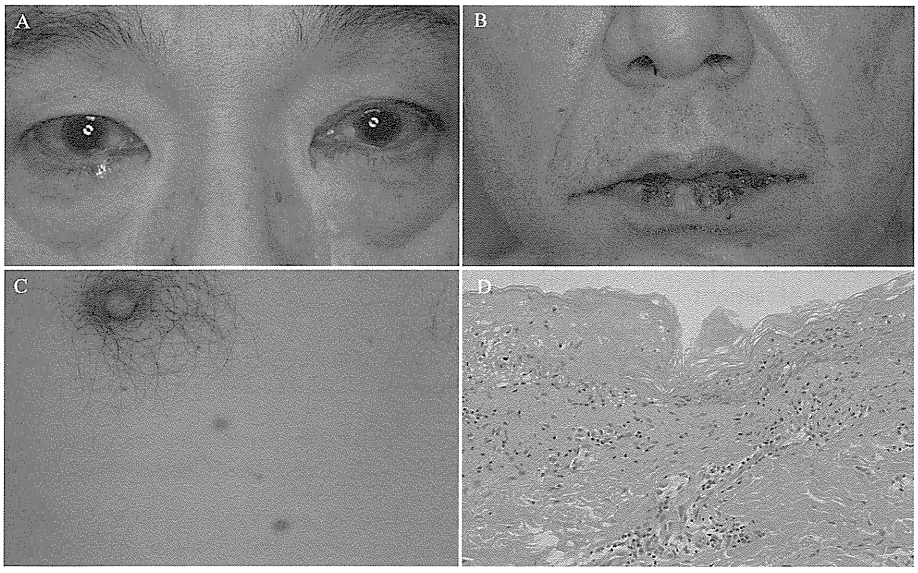

Fig. 1. Case 2 (A) Bilateral conjunctivitis with pseudomembranous formation. (B) Haemorrhagic scales on the lip. (C) Several scattered macules with bullae on the trunk. (D) Epidermal necrosis with a mild lymphocytic infiltration in the dermis (haematoxylin and eosin (H&E) stain; original magnification ×100).

 doi: 10.2340/00015555-2180
 ISSN 0001-5555

X-ray disappeared. The LTT for loxoprofen became negative 6 months after disease onset (Table SI[1]). This case has been described in part elsewhere (8).

At the 1-year and 2-year follow-up of patients 1 and 2, respectively, no sequelae were detected.

DISCUSSION

In adult SJS, drug reactions, rather than infections, have been emphasized as the main causative agent; therefore, intensive investigation for the culprit drug is carried out (9). The co-involvement of infectious agents might be overlooked in the clinical setting in adult patients with SJS. In children, Mp infection has been postulated as the most common implicated factor for the development of SJS (10). The characteristics of paediatric SJS associated with Mp infection are severe mucocutaneous involvements, such as oral ulcers, and keratoconjunctivitis, in the absence of skin lesions. In this regard, atypical SJS cases have been reported as Fuchs syndrome (4, 11), Mp-associated mucositis (12, 13), and incomplete SJS (14).

The clinical characteristics of our 2 patients were similar to those observed in paediatric SJS associated with Mp infection. The results of our serological examination showed significant alterations in Mp antibody titres in the 2 cases, with infiltrative shadowing on the X-ray in 1 case. Thus, it is clear that the preceding Mp infection contributed to the development of SJS in these 2 cases. In addition, drugs were given before the appearance of mucosal lesions in these 2 cases. LTT was performed to determine the causative drug and the results were positive in both cases. Therefore, the involvement of drug reactions was suspected in our atypical SJS adult patients. Although challenge tests could not be performed because the Committee of Severe Cutaneous Adverse Drug Reactions advises against the use of these tests, no positive LTT levels for diclofenac or loxoprofen in healthy individuals were observed with our method, which supports the involvement of a drug reaction in the present cases. Although LTT levels cannot predict whether sensitization leads to clinical symptoms, it has been shown that strong immune reactivity is frequently associated with clinical symptoms (6), and LTT needs to be carried out at the acute stage of SJS to avoid false-negative results (7). In the present cases, LTTs were performed at the acute stage. Therefore, it is likely that the interaction between Mp infection and drug reaction with the involvement of drug-specific T cells might have played an important role in the appearance of SJS in both patients.

Although the involvement of Mp infection in the appearance of SJS has not been clearly shown, Mp infection might affect the immune response in the initial stage of development of SJS, thereby contributing to the atypical clinical manifestations of SJS. It remains unknown why SJS lesions in Mp-infected patients are confined to the mucous membranes in the immune-mediated process. The limited severe inflammatory cellular infiltration around the sites of infection might have prevented dissemination of the pathogens. In addition, molecular mimicry, with similarities between Mp proteinaceous adhesions and certain specific antigens in mucous membranes, has been hypothesized (1).

On the other hand, we showed previously that regulatory T cells (Treg) are profoundly impaired in SJS compared with those in other severe drug eruptions, such as drug-induced hypersensitivity syndrome (15). Given that Tregs recognize mycoplasma through toll-like receptor (TLR)-2 and modify their function, the suppressive function of Treg might be temporarily impaired in association with Mp infection (16, 17). The preceding Mp infection might provide a favourable milieu for the expansion of drug-specific effector T cells, thereby facilitating drug reactions despite the short-term drug administration. In support of this, the positive LTT that was performed at the initial stage of the disease became negative after complete resolution of the illness in the second case.

ACKNOWLEDGEMENTS

This work was supported in part by Research on Measures for Intractable Diseases Project matching fund subsidy from the Ministry of Health, Labor and Welfare, and by the Ministry of Education, Culture, Sports, Science and Technology (to TS and YK).

The authors would like to thank Dr K. Matsuda and S. Matsuda from M Bio Technology Inc. for providing information about *Mycoplasma* antibody measurements.

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Clinic Rev Allerg Immunol (2015) 49:192–202
DOI 10.1007/s12016-014-8421-3

Crucial Role of Viral Reactivation in the Development of Severe Drug Eruptions: a Comprehensive Review

Tetsuo Shiohara · Yukiko Ushigome · Yoko Kano · Ryo Takahashi

Published online: 16 April 2014

Abstract A growing number of cells, mediators, and pathways have been implicated in severe drug eruptions. Fifteen years ago, we published landmark studies that sparked the current advances in our understanding of the role of viral reactivations in severe drug eruptions. Viral reactivations then became critically important as diagnostic tools, but how precisely they participated in the pathogenesis remained less well-defined. The question of whether viral reactivations are pathogenic or are instead as epiphenomenon of severe tissue damage has plagued the field of drug allergy for some decades. Recent evidence points to a crucial role for tissue-resident memory T (TRM) cells in immune protection against viral infections. Yet immune protection against viral infections is but one side of a coin, the other side of which comprises effector cells capable of mediating severe immunopathology: Once drug antigen is cross-recognized by these T cells, they could be activated to kill surrounding epidermal cells, resulting in drug-induced tissue damage. Such TRM cells could persistently reside in the skin lesions of fixed drug eruptions (FDE) and are most likely a major cell type responsible for the development of FDE. We also discuss the role of regulatory T (Treg) cells in the setting of drug allergy, in which herpesviruses are reactivated in sequence. Although many details of the complicated interactions among viruses, anti-viral immune responses, TRM cells, and Treg cells remain to be elucidated, we review the current status of this rapidly advancing field.

T. Shiohara · Y. Ushigome · Y. Kano
Department of Dermatology, School of Medicine, Kyorin University, Mitaka, Tokyo 181-8611, Japan

T. Shiohara · R. Takahashi (✉)
Division of Flow Cytometry, School of Medicine, Kyorin University, Mitaka, Tokyo 181-8611, Japan
e-mail: ryo@ks.kyorin-u.ac.jp

Keywords Regulatory T cells · Herpesviruses · Resident memory T cells · Fixed drug eruption · Drug-induced hypersensitivity syndrome · Immune reconstitution syndrome · Graft-versus-host disease

Introduction

The long-standing question of why drug allergy develops in limited numbers of susceptible individuals who take drugs is still largely unresolved. Many studies have addressed this question: Clinical observations have indicated that drug allergy is often precipitated by viral infections [1, 2]. According to the viral hypothesis, viral infections could predispose genetically susceptible individuals to the subsequent development of drug allergy [3, 4]. The list of viruses triggering or exacerbating drug allergy in susceptible individuals is constantly growing and includes Epstein–Barr virus (EBV), herpes simplex virus (HSV), human herpesvirus 6 (HHV-6), cytomegalovirus (CMV), and varicella-zoster virus (VZV) [5–7]. Fifteen years ago, we [7] and Dr. Hashimoto's group [8] independently published landmark studies that sparked the current advances in our understanding of the role of viral infections in drug allergy. These initial studies have detected HHV-6 DNA by polymerase chain reaction (PCR) in blood and skin specimens from patients with a certain type of drug allergy over a predictable time course, namely 2–3 weeks after onset. However, because HHV-6 detection by PCR was limited to convenience blood samples obtained 2–3 weeks after onset of the drug allergy, there are difficulties in assigning the causality of drug allergy to the virus. What, then, is the meaning of the virus detection at 2–3 weeks after onset of the drug allergy? These findings could be interpreted as indicating the possibility that viruses are involved in acute exacerbations of drug allergy but not in the induction. On the other hand, there is also the growing body of evidence that drug allergy can be

profoundly influenced by viral infections that occurs before onset of drug allergy, as exemplified by ampicillin rashes in infectious mononucleosis (IM) [1]. Alternatively, virus infection may be additional event that is required for drug sensitization to progress to drug allergy. Thus, the complexity of assigning a pathogenic role to any virus in the development of drug allergy is underscored by the available evidence that the severity and clinical course of drug allergy can be influenced by viral infections that occur before, concurrent with, or subsequent to drug allergy. In this review, we focus primarily on how viral infections and virus-driven immune responses can evoke drug-specific immune responses that are presumably capable of eliciting cell and tissue damage.

Tissue Localization of Resident Memory T Cells After Infection with HSV

Elucidation of the events leading to clearance of infected viruses from skin could be key to our understanding of how a drug-specific immune response can develop after viral infection. Recent studies have clearly shown that after the clearance of viral infection such as HSV, a small fraction of memory T cells persist as a stable population to confer protection upon reencountering the same virus in peripheral tissues such as skin [9–12]. These HSV-specific T cells persist in the skin for at least 6 months after infection with HSV and express CD8, VLA-1, and CD103, molecules important for epithelial localization. These $CD8^+$ T cells, defined as tissue-resident memory T (TRM) cells, are different from $CD8^+$ T cells of the central memory phenotype (TCM) that largely recirculate between the secondary lymphoid organs, in that TRM cells are resident in the epidermis and are confined largely to the original site of infection [9, 10]. These $CD8^+$ TRM cells are phenotypically distinct from TCM cells with low expression of CD62L and CD122 but high expression of CD69 [9]. According to a recent report [11], they show a steady-state crawling behavior in between keratinocytes, and their migratory dendritic behavior allows the detection of antigen-expressing target cells in physiologically relevant time frames of minutes to hours. Interestingly, these $CD8^+$ TRM cells in distant skin sites markedly have been shown to reduce viral loads to levels comparable to those observed at the actual site of previous infection [12]. These $CD8^+$ TRM cells produce effector cytokines such as IFN-γ, persist at the site of infection for many months, and are highly effective at rapidly eliminating virus from the skin. After viral infection through the skin, these $CD8^+$ T cells distribute not only to the site of infection but also throughout the entire skin surface [12], providing long-lived protective T cell immunity against re-infection of the virus. Although these virus-specific $CD8^+$ TRM cells are also found in sensory ganglia, brain, intestinal mucosa, and salivary glands, common features of these differentially localized TRM cells are the expression of CD103 and of CD69. The salivary glands as well as the skin also harbor virus-specific $CD8^+$ TRM cells uniquely expressing E-cadherin at surprisingly high frequencies after systemic virus infection [13].

Thus, virus-specific $CD8^+$ TRM cells resident in the skin site are thought to regulate whether skin infection with the virus could result in viral control, asymptomatic persistence, or severe pathology. As demonstrated by Jiang et al. and Mackay et al. [12, 14], these skin-resident CD8 TRM cells are long-lived and non-recirculating and are superior to circulating TCM cells at providing rapid long-term protection against cutaneous viral infections ever in the absence of persisting local antigen presentation. Depending on the viral loads in the skin site, virus-specific TRM cells resident in the skin site could have either a beneficial or detrimental role in controlling virus-associated morbidity: At a medium dose of virus, TRM cell-mediated lysis of virus-infected cells contribute to sufficient control of viral burden, while, at a high dose of virus, TRM cells would act detrimentally by severely damaging virus-infected epidermal cells, ultimately resulting in severe T-cell-dependent immunopathology.

$CD8^+$ TRM Cells in the Lesions of Fixed Drug Eruption

The classic fixed drug eruption (FDE) lesions are characterized by a solitary or small number of well-circumscribed, round, and/or oval erythematous macules and plaques with dusky centers on the skin and/or mucous membrane: These lesions usually start abruptly an exactly the same site with each administration of the causative drug [15, 16]. Although the individual FDE lesions are 1–4 cm in diameter and rarely exceed 10 cm, these lesions become more numerous and more severe unless the causative drug is withdrawn. New FDE lesions often develop at the site of viral infection such as HSV and previously traumatized or inflamed skin such as insect bites, burn, and venipuncture sites [17]. A peculiar linear pattern of FDE lesions suggestive of previous herpes zoster (HZ) have been also reported, although it is unclear whether the patient had preceding HZ before onset of FDE [18]. An unusual cellulitis-like FDE has also been reported: An erythematous and edematous plaque with undetermined borders mimicking cellulitis was elicited by the subsequent administration of the causative drug at the same sites [19]. These findings, together with our previous report describing the development of typical FDE lesions at exactly the same site as the patient's previous HSV lesion, suggested to us the possibility that cells with "protective" function may be recruited from the circulation, either nonspecifically or specifically, upon primary insults, such as trauma and viral infections, and they could persist at relatively high frequencies in the lesional

skin and be responsible for the subsequent induction of FDE lesions (Fig. 1). Indeed, innate immune cells such as dendritic cells or $\gamma\delta^+$ T cells and antigen-specific $CD4^+$ and $CD8^+$ T cells are shown to be recruited from the circulation to the inflammatory site such as skin and persist in the epithelium in a number of diverse physiological and pathological settings.

To investigate the possibility that $CD8^+$ TRM cells could persist in FDE lesions, we immunohistochemically characterized resting FDE lesions long after clinical resolution. FDE lesions typically resolve after discontinuation of the causative drug, leaving hyperpigmentation localized to the sites of previous flare. Such resolved FDE lesions are characterized by a small number of $CD3^+$ $CD8^+$ T cells aligned along the epidermal site of the dermoepidermal junction: These T cells persist for a long time in the lesion, referred to as resting FDE lesions, after resolution as a phenotypically homogenous, stable population of T cells that constitutively express TCR-αβ, CD45RA, CD103, CLA, CD11b, CD69 but not CD27 and CD56 [15]. In contrast, these T cells are rarely found in the uninvolved epidermis of FDE patients and healthy individuals. This phenotype of T cells most closely resembles that of TRM cells. Our previous studies demonstrated that the $CD8^+$ T cells isolated from the resting FDE lesions and subsequently expanded in vitro displayed cytolytic activity against NK-sensitive or NK-resistant tumor cells and cultured keratinocytes when stimulated in an Ag-nonspecific fashion via CD3/TCR complex [20]. Nevertheless, they are not constitutively cytolytic, unlike NK cells and murine $\gamma\delta^+$ dendritic epidermal T cells (DETC). The intracellular cytokine assay with the use of $CD8^+$ T cells freshly isolated from the resting FDE lesions showed that the great majority (>80 %) of these $CD8^+$ T cells produced IFN-γ and TNF-α upon stimulation while the proportion of these T cells producing IL-4 was very low (<1 %). Our in situ PCR studies using FDE lesions obtained 3 h after challenge demonstrated that these $CD8^+$ TRM cells could be induced to express IFN-γ mRNA and protein upon clinical challenge with the causative drug [21]. Their induction of IFN-γ mRNA was much faster than that of their dermal and peripheral counterparts. Because their rapid production of large amounts of IFN-γ mRNA and protein upon stimulation with the causative drug in vivo was clearly followed by localized epidermal damage, these $CD8^+$ T cells residing in resting FDE lesions are most likely a major cell type responsible for the development of FDE [16, 21].

Despite our expectation, however, no convincing evidence is presently available to indicate that the ligands for these $CD8^+$ TRM cells resident in the resting FDE lesions are drug antigens or viral antigens. In this regard, our previous studies demonstrated that some of these $CD8^+$ TRM cells can recognize self-proteins [16] but not drug antigens either in a totally major histocompatibility complex (MHC)-dependent or MHC-independent fashion. However, in view of our previous quantitative PCR analysis demonstrating that these $CD8^+$ TRM cells utilized a very limited range of TCR Vα and Vβ gene families as compared with peripheral blood T cells obtained from the same patients [20], we can assume that they can recognize a limited Ag presented by MHC molecules. In this regard, it is noteworthy that a recent report indicates that heterologous virus infections of mice result in a narrow oligoclonal TCR repertoire specific to highly cross-reactive epitopes of different viruses [22]. What causes narrowing of the TCR repertoire in mice following heterologous virus infections remains poorly understood, but the profound narrowing of the TCR repertoire diversity after heterologous virus infections is likely a consequence of expansions of the highly cross-reactive T cell population. Thus, cross-reactivity of $CD8^+$ T cells generated after heterologous virus infections may explain why FDE lesions can be induced at exactly the same site as the patient's previous HSV infection or trauma. Such cross-reactivity of $CD8^+$ TRM cells resident in the epithelium may help to control a variety of pathogens early in infection. The most likely explanation for why $CD8^+$ TRM cells originally distributed to the site of infections can be activated by totally unrelated drug antigens is that these $CD8^+$ TRM cells could be broadly cross-reactive with some

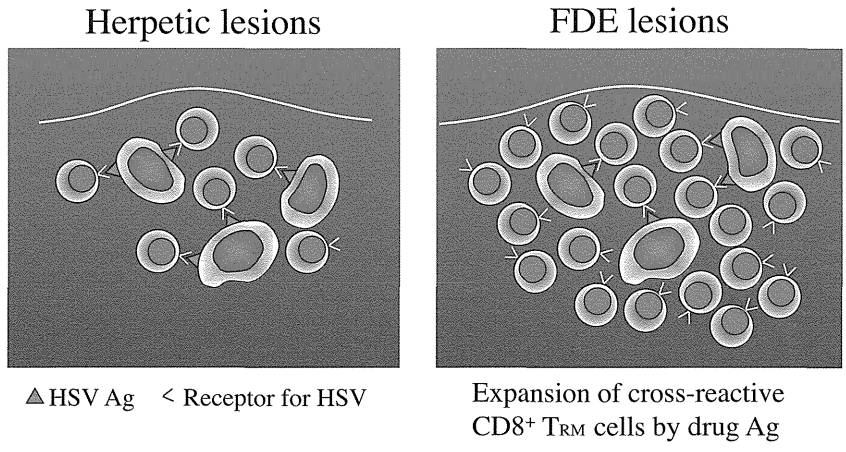

Fig. 1 HSV lesions evolve into FDE lesions. After infection with HSV, a small fraction of $CD8^+$ TRM cells specific for HSV persist as a stable population with antigen-presenting cells at the skin site of HSV infection to confer protection against the same virus. These T cells, once activated with cross-reactive drug antigen, can expand and become effector cells responsible for the induction of FDE

of drug antigens while preserving the fine specificity for a self-MHC-bound peptide such as viral antigen. In support of this possibility, there is now sufficient evidence to indicate that the specificity of a large proportion of antigen-specific self-HLA restricted T cells is also directed toward infectious agents, particularly herpesviruses [23–25]. Additional mechanisms for how cross-reactivity of T cells can be maintained in vivo have been reported in recent studies [26, 27]: These cross-reactive T cells can recognize self- and nonself HLA molecules while maintaining a strong antiviral immune response by recruiting non-cross-reactive T cells to control the virus. Thus, $CD8^+$ TRM cells enriched in resting FDE lesions could have originally evolved to protect epidermal tissue integrity from invading pathogens such as herpesviruses, and once drug antigen is cross-recognized by these T cells because of their broad cross-reactivity, they can be activated to kill surrounding keratinocytes, resulting in localized epidermal damage [15].

Role of Regulatory T Cells in FDE Lesions

The clinical spectrum of FDE is highly variable, ranging from the classic form to a generalized bullous variant with systemic symptoms initially indistinguishable from Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). Despite such clinical similarities between a generalized form of FDE and SJS/TEN, subsequent evolution of the two conditions is quite different: The former resolves spontaneously upon discontinuation of the causative drug, while the latter often results in full-thickness epidermal detachment, rapidly spreading to the whole body. However, less is known about critical events that are needed for preventing further disease progression to SJS/TEN. In this regard, our previous studies demonstrated that recruitment of $FoxP3^+$ regulatory T (Treg) cells into the FDE lesions is crucial for preventing $CD8^+$ TRM and TCM cells from excessively activating at the inflammatory site [28]. These observations suggested that the defect in regulatory mechanisms for preventing further progression to SJS/TEN may reside either within the cutaneous milieu in the inflammatory site, particularly in the border of the lesion, or within migrating Treg cells themselves; this is because the individual erythematous lesions of FDE have well-defined border while the SJS/TEN lesions form poorly defined macules rapidly extending to the perilesional skin.

Our recent unpublished study has demonstrated that $FoxP3^+$ Treg cells obtained from FDE patients at the acute stage retain the suppressive capacity to inhibit proliferation of $CD8^+$ TRM and TCM cells while their function in SJS/TEN patients at the corresponding stage is severely impaired [29], indicating that Treg cells in FDE are fully functional and constitute an important component of protective immunity. We have further demonstrated that $FoxP3^+$ Treg cells preferentially accumulate beneath the epidermis and at the mid part of the dermis in the periphery of the FDE lesions while those are sparsely distributed in the upper part of the dermis of the periphery of SJS/TEN lesions. These results indicate that timely and selective accumulation of Treg cells in the periphery of FDE lesions could be crucial for preventing excessive activation and recruitment of $CD8^+$ TRM and TCM cells. Indeed, the frequency of Treg cells in the periphery of FDE and TEN lesions correlated well with the degree of protection conferred. These findings emphasize the importance of Treg cell recruitment to the extending edge of the inflammatory site for establishing the Treg response to the greater load of infiltrating Teff cells. We also provide evidence to indicate that mast cells accumulating in the FDE lesions may facilitate the rapid recruitment of Treg cells to the inflammatory sites thereby limiting tissue damage mediated by activation of $CD8^+$ TRM and TCM cells. Consistent with these data, mast cells and Treg cells have been shown to exhibit substantial colocalization in tissues and lymph nodes. Because IL-16 able to attract Treg cells was much more intensely expressed in mast cells detected in the FDE lesions and IL-16 was the only cytokine that increased rapidly in the serum of patients with FDE after clinical challenge (Y Mizukawa et al., unpublished data), we conclude that a timely and proper localization of Treg cells into the specific inflammatory site induced by mast cell-derived IL-16 in the FDE lesions could serve to limit excessive activation of potentially destructive $CD8^+$ TRM and TCM cells, resulting in spontaneous resolution of the FDE lesions.

The Effect of Viral Infections on the Subsequent Development of Drug Allergy

Available evidence strongly suggests that viral infections create a favorable milieu for the initiation and progression of adverse drug reactions [4]. It remains unknown, however, how preceding viral infections induce or contribute to the subsequent development of adverse drug reactions. When considering a complex interaction between viral infection and drug allergy, it is noteworthy that there must be mechanisms that protect the host from excessive immune responses to viruses, which could in themselves lead to greater pathological consequences than the invading viruses. Evidence is recently accumulating that $CD4^+FoxP3^+$ Treg cells, either natural or inducible, can inhibit the function of effecter T (Teff) cells at the site of viral infections, thereby inhibiting severe immunopathology. On the other hand, the Treg response may be potentially harmful to the host in terms of infection control because their activation and expansion secure survival of invading viruses for an extended period of time, thereby causing chronic infectious diseases. Numbers and function of Treg cells, therefore, should be controlled depending on the stage of viral

infections. During the early stage of infection, dampening Treg function would result in vigorous anti-viral responses that control infections. Some studies have demonstrated that Treg cells lose their suppressive capacity in response to engagement of virus-sensing mechanisms such as TLR signaling [30]. Alternatively, it has been proposed that, during viral infection, TCM and TRM cells responding to infection would become resistant to Treg-mediated suppression as a result of exposure to proinflammatory cytokines and increased costimulatory signals [31]. At later time points in infection, however, expansion of functional Treg cells is likely to occur to protect overstimulation of the immune system. Thus, a time-dependent balanced, rather than biased, Treg responses would be necessary for host protection and the resolution of infection. One must appreciate, however, the fact that most of previous studies on the role of Treg cells in the setting of viral infections were not extended beyond the acute period of infection to determine how Treg cells were involved in the pathogenesis of virus-induced diseases.

To investigate the role of Treg cells in the context of viral infections, we initially evaluated the frequencies of $CD4^+CD25^+FoxP3^+$ Treg cells in total PBMC of patients with viral infections, such as VZV and parvovirus B19. Although recent studies demonstrated an increase in Treg frequencies in acute dengue [32] and measles infection [33], our results showed no significant alterations in Treg frequencies and their absolute numbers in the setting of these viral infections. These apparently conflicting results suggest that there is more to be learned about the frequency of Treg cells during acute infections: The number of Treg cells during viral infections would be different depending on the virus, virulence, or dose. More importantly, we demonstrated that Treg cells obtained from patients with these viral infections, VZV and parvovirus B19, exhibited a significantly impaired capacity to suppress CD3-driven Teff cell proliferation, as compared with those from healthy controls. The degree of functional defect in patients at the acute stage of these viral infections was comparable to that in patients with TEN, which was previously described by us [29]. Their impaired capacity at the acute stage of these viral infections, however, had returned to a presumed baseline, which was indistinguishable from that of healthy controls, upon clinical resolution. The defect during the acute stage was not due to increased resistance of Teff cells obtained from these patients to Treg-mediated suppression. In contrast, functional activity of the Treg cells obtained from patients with Mycoplasma pneumoniae (MP) remained defective even 1 year after clinical resolution (R Takahashi et al., manuscript submitted). These results indicate that defective Treg function observed during the acute stage of the viral infections and both the acute and resolution stages of MP infections would serve to lower the activation threshold of drug-specific T cells or pathogen-specific T cells, thus facilitating the development of drug allergy. In these viral infections, a loss of Treg function was transient and the defective Treg cells regained their functional competence upon resolution, while MP infection persistently abrogated Treg functions even after clinical resolution. These results provide an explanation for why patients with MP-associated SJS displayed polysensitivity to multiple drugs with different structures that cannot be easily explained by drug antigen-driven T cell activation [34]. Thus, viral or MP infections are likely to be prime candidates for subsequently developing drug allergy in susceptible individuals, probably through a transient or persistent loss of Treg functions.

Viral Reactivation in Drug-Induced Hypersensitivity Syndrome

Several drug eruptions encompass several distinct clinical entities, the most serious being TEN/SJS. Drug-induced hypersensitivity syndrome (DiHS), also referred to as drug reaction with eosinophilia with systemic symptoms (DRESS), represents the opposite end of a spectrum of severe drug eruptions. DiHS/DERSS offers a unique opportunity to link between viral infections and the development of severe drug eruptions, due to its strong association with HHV-6 infection [7, 8]. This syndrome has several unique features that cannot be solely explained by a drug Ag-driven, oligoclonal T cell activation: The delayed onset in relation to the introduction of the causative drug is one of the important features of this syndrome that can be distinguished from other types of drug eruptions, which usually start 1–2 weeks after starting therapy. This syndrome typically occurs with fever and cutaneous lesions 3 weeks to 3 months after starting therapy with a limited numbers of drugs, mainly anticonvulsants. Importantly, more severe reactions often occur 3–4 days after withdrawal of the causative drugs: This paradoxical worsening is also characteristic of DiHS and may be mistaken for severe infectious diseases. Patients with DiHS often show unexplained cross-reactivity to multiple drugs with different chemical structures, including those starting after onset of symptoms. In addition, variable clinical symptoms, such as renal and liver symptoms, continue to deteriorate one after another even for weeks after stopping the causative drug. Although maculopapular or erythematous eruptions are initially observed on the face, upper trunk, and upper extremities, most erythematous macules do not evolve into blisters and no mucous membrane involvement is usually seen [15, 35, 36].

The peripheral blood usually shows marked leukocytosis with atypical lymphocytosis or eosinophilia of various degrees in most of cases, although in some cases leucopenia or lymphopenia may precede the leukocytosis. A dramatic decrease in serum IgG, IgA, and IgM levels is typically observed at onset, and the lowest levels are usually seen a week after withdrawal of the causative drug. Despite such variable

clinical presentations and courses, HHV-6 reactivations can be detected at a particular time point, 2–3 weeks after onset of rash in the vast majority of patients regardless of treatment [15, 36]: A strong association between HHV-6 reactivations and this syndrome has been supported by a large number of independent groups over the years in Japan [36, 37]. This is the reason why HHV-6 reactivations as evidenced by the rise in anti-HHV-6 IgG titers and HHV-6 DNA levels can be used to confirm a clinical diagnosis of DiHS [38]. Although HHV-6 was initially thought to be the only virus reactivated during the course of DiHS, recent studies of real-time measurements for viral loads have demonstrated that other herpesviruses, such as EBV, HHV-7, and CMV, are also reactivated in sequence during the course of the disease as demonstrated in graft-versus-host diseases (GVHD) [35, 39] (Fig. 2). According to our sequential analysis of viral loads in patients with DiHS, the cascade of reactivation events initiated by HHV-6 or EBV would extend, with some delay, to HHV-7 as well and eventually to CMV [39] (Figs. 2 and 3). Consistent with the previous observations that the severity of GVHD was correlated with the levels of HHV-6 DNA [40], the magnitude of HHV-6 reactivation as evidenced by the increase in HHV-6 DNA levels was correlated well with the severity of inflammatory responses that occur in vivo in patients with DiHS [15, 41]. These findings provide strong evidence to suggest the role of HHV-6 or other herpesviruses in the etiology of the disease, rather than a mere bystander, although reactivation of these viruses as a result of a transient immune dysfunction cannot be definitely excluded. Because of the unique biological properties of herpesviruses, particularly their "immunotropic" nature, and their possible interactions with other herpesviruses, they may have detrimental effects on the immune system once reactivated in the course of the disease. Investigators have been hampered by difficulty in assigning a pathogenic role to any herpesvirus in patients with DiHS who manifest clinically variable symptoms in different organs. Thus, despite rapid advances in the biology and genetics of herpesviruses, progress in understanding the pathogenic role of these herpesviruses has not come easily.

How, then, can the etiological role of herpesviruses be confirmed? One relevant observation from years of research on the role of immune responses against EBV is that cutaneous and visceral symptoms of DiHS/DRESS are mediated by activated $CD8^+$ Teff cells which are largely directed against herpesviruses, such as EBV, and that the causative drug can reactivate herpesviruses in vitro [42]. The result of this study indicates the possibility that herpesvirus reactivations triggered by the causative drug could have the immunopathogenic role in DiHS/DRESS but not a mere epiphenomenon of the underlying immunodeficiency.

Role of Treg Cells in DiHS

Our recent study clearly demonstrates that the acute stage of DiHS/DRESS is characterized by dramatic expansions of fully functional $CD4^+FoxP3^+$ Treg cells while their suppressive capacity is profoundly impaired in the acute stage of SJS/TEN [43]. Although it is difficult to determine when Treg expansions occur before the development of DiHS, this expansions of Treg cells would occur far before onset of DiHS, which would contribute to not only the delayed onset but also to viral reactivations [36]. In order to counterbalance activating Teff cells, expansions of Treg cells are likely to be key for maintaining a healthy balance between protection and immunopathology. However, once the balance has been disturbed toward activation of Teff cells, DiHS ensues (Fig. 4). Thus, the expanded Treg cells would also limit the severity of Teff cell-mediated immunopathology, which is reflected by the observation that epidermal damage can be rarely detected in the

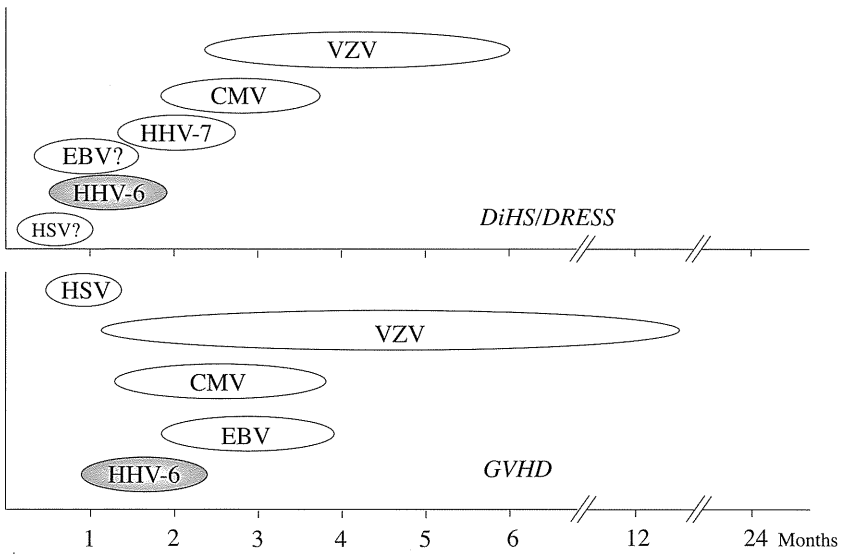

Fig. 2 Sequential reactivation of various herpesviruses during the courses of DiHS and GVHD. Importantly, herpesviruses are reactivated in DiHS in the fundamentally same order as in GVHD

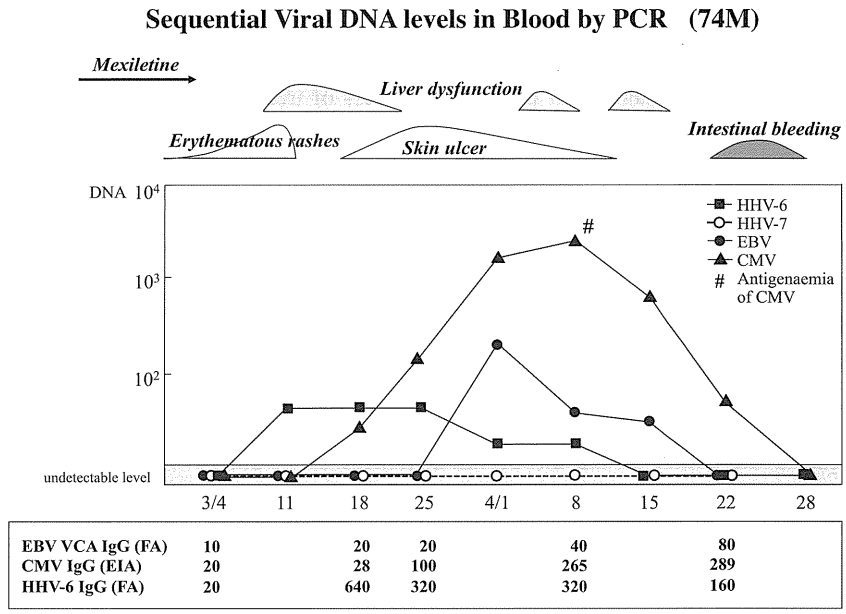

Fig. 3 Relation between clinical symptoms and herpesvirus viral DNA loads and anti-viral antibody titers in a representative case with DiHS due to phenobarbital

skin lesions of DiHS. The expanded population of Treg cells in the peripheral blood of DiHS patients during the acute stage is likely the inducible Treg (iTreg) cells that are induced in the periphery under specific conditions of cytokine and antigen [43]. iTreg cells can be produced from $CD4^{+}CD25^{-}$ T cells by culture with antigen and TGF-β or IL-10 and TGF-β, while IL-6 inhibits iTreg induction and promotes Th17 [44, 45]. Consistent with this view, our preliminary study shows that in vitro culture with the causative drug of peripheral blood lymphocytes from DiHS patients after resolution results in expansions of Treg cells (unpublished observation). Importantly, a gradual loss of Treg-cell function occurs after the resolution of DiHS, although it remains unknown when and how it occurs: Expanded Treg cells, upon their contraction, may become functionally exhausted and loss their essential functional activity necessary for immune protection. Such functional exhaustion is likely to result from repeated activation by Treg cells frequently occurring during the courses of DiHS and is a way of limiting the magnitude of Treg cell responses, which may compromise effective immunity against infections agents. Indeed, reflecting a loss of Treg-cell function after resolution, several autoimmune diseases such as type 1 diabetes mellitus, thyroiditis, SLE, and sclerodermoid GVHD-like disease [46] have been reported to develop at intervals of several months to years after clinical resolution of DiHS [35, 47] (Fig. 5). In view of the finding that the imbalance between the Treg and Teff-cell compartments has been shown to trigger the development of autoimmune disease, resolution of DiHS may be accompanied by a shift away from Treg differentiation and toward IL-17-

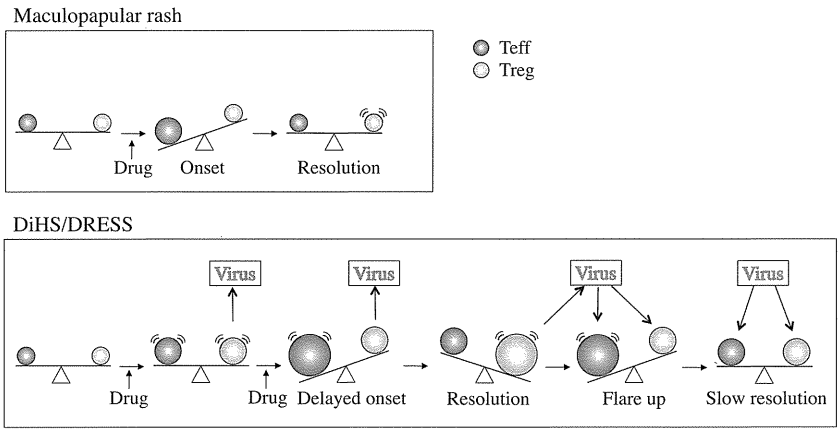

Fig. 4 A hypothetical model for the development of DiHS and maculopapular type of drug eruption. In maculopapular rash, Treg cells are overwhelmed with expansions of Teff cells. In DiHS, protracted use of the causative drug results in expansions of not only Teff but also Treg cells, thereby delaying onset and causing viral reactivation. Eventually, however, the delicate balance between Teff and Treg is disturbed toward activation of Teff cells, leading to onset of DiHS. Systemic corticosteroids can improve clinical symptoms, probably by potentiating Treg-cell function

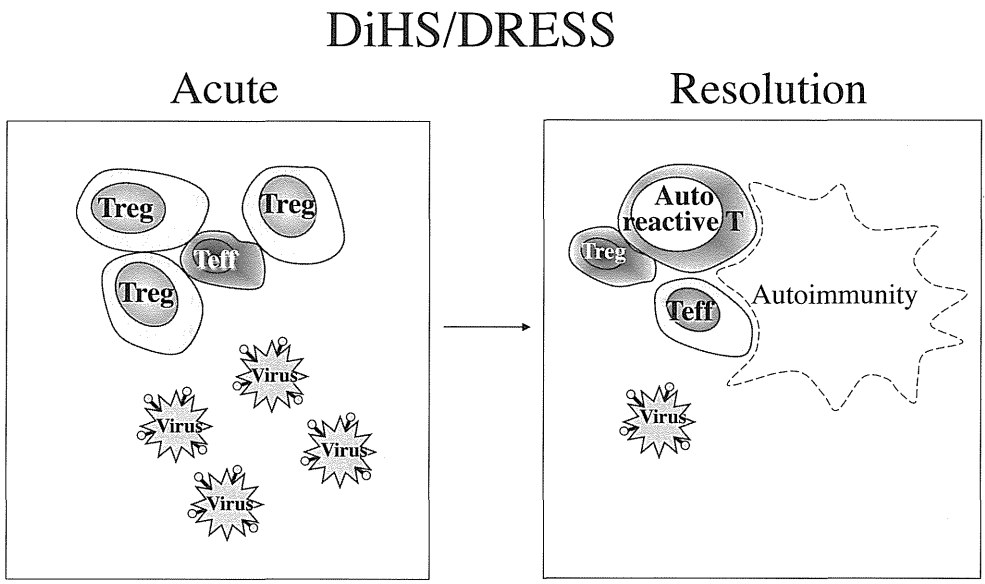

Fig. 5 Acute and resolution stages of DiHS. After resolution of clinical symptoms, Treg cells expanded during the acute stage are contracted and become exhausted in function, thereby triggering autoimmune diseases

producing (Th17) cell differentiation. We therefore measured the frequencies of Treg cells and Th17 cells within circulating $CD4^+$ T cells during the acute stage and again long after resolution. We found that Th17 cells were increased in frequency coincident with the decrease in Treg cell frequency upon resolution in DiHS. A significant increase in various autoantibody titers such as anti-nuclear antibody (ANA) and anti-thyroglobulin antibody was specifically observed in patients with DiHS after resolution, which likely reflects a shift to Th17 cell differentiation (unpublished data).

Longitudinal Analyses of Herpesvirus Loads in Severe Drug Eruptions

Although sequential reactivations of several herpesviruses have exclusively been demonstrated during the acute stage of DiHS, no previous studies were extended beyond the acute stage of the stage. We therefore sought to investigate whether herpesvirus reactivations could be observed in SJS/TEN and beyond the acute stage of both diseases. EBV, HHV-6, and CMV DNA loads were sequentially determined during a 2-year period after onset. Our quantitative PCR analysis revealed persistently elevated EBV loads in patients with SJS during the acute stage and long after clinical resolution [48]. In contrast, only a fraction of patients with DiHS/DRESS had increased levels of EBV DNA in the blood at onset. In many patients with SJS, increased EBV DNA persisted for up to 2 years after resolution. These results suggested that patients with high EBV DNA loads may be at risk of subsequently developing SJS, although we could not totally exclude the alternative possibility that the aggressive clinical course observed during the acute stage of SJS may be responsible for EBV reactivations. However, this alternative possibility is unlikely because the degree of the EBV loads in patients with SJS did not correlate with the severity of clinical symptoms and laboratory abnormalities. Surprisingly, we noted that no patients with TEN demonstrated elevated EBV loads during the acute stage and after clinical resolution [48]. In view of clinical similarities between SJS and TEN, differences in the pattern of the viral loads between them were surprising and could be interpreted as indicating the possibility that these two diseases may be distinct in the pattern of persistent viral infections although they may share important common pathophysiologic processes [49].

Increased EBV, CMV, and HHV-6 loads only occurred during the acute stage and a post 100-day period in patients with DiHS/DRESS [48]. Nevertheless, the dynamics of EBV, CMV, and HHV-6 reactivation varied considerably in these patients according to the use of systemic corticosteroids. Although CMV and HHV-6 DNA loads were higher in those receiving systemic corticosteroids than those not receiving them, EBV DNA loads were significantly higher in those without them [48]. Interestingly, the increase in various autoantibody titers, which was detected 1 year after the resolution of DiHS/DRESS, was associated with the elevated EBV loads during the acute stage of DiHS/DRESS and preferentially observed in patients not receiving systemic corticosteroids [50]. These results could be interpreted as indicating that the use of systemic corticosteroids during the acute stage of DiHS may serve to prevent the progression to autoimmune disease as long-term sequelae of DiHS/DRESS, probably through the decrease in EBV DNA loads. Similar observations have been also noted in the generation of autoantibodies to epidermal proteins, periplakin (unpublished data). Consistent with the results of autoantibodies such as ANA, the generation of autoantibodies to periplakin was preferentially observed in patients with DiHS/DRESS who were not treated with systemic corticosteroids. These findings suggest that immune responses

preventable with systemic corticosteroids and/or increased EBV DNA loads could trigger the subsequent generation of autoantibodies to periplakin and that early resolution by systemic corticosteroids may lead to better long-term outcomes for patients at risk of subsequently developing autoimmune disease.

Management of Patients with Severe Drug Eruptions Associated with Viral Reactivations

Physicians, when treated patients with severe drug eruptions, need to be aware of underlying viral infections, particularly herpesvirus infections, as one of the most important aspects of management of these patients. Because those patients often receive immunosuppressive agents either early or later in the course of their illness, a wealth of information on the interaction between herpesviruses and immune responses should be gathered to better manage those patients.

When we consider how to better manage those patients, one must appreciate the concept of immune reconstitution syndrome (IRS) [51–53]. IRS is an increasingly recognized disease concept and is observed with a broad spectrum of immunosuppressive therapy-related opportunistic infectious diseases and severe drug eruptions complicated by viral reactivations. Increased occurrence of opportunistic infections associated with defects in the immune system was generally recognized as a result of microbial damage afflicted by these pathogens. Contrary to this belief, an intriguing aspect that has received little attention so far is that restoration of hose immunity may also have adverse sequelae, particularly when it occurs abruptly and rapidly. Indeed, when the timing of onset of an adverse event was carefully assessed in patients infected with HIV, the onset of this event was concentrated within 6–14 days of starting antiretroviral therapy (ART) [54], coincident with restoration of host $CD4^+$ T cell number and reactivity. This clinical deterioration observed after starting HAART therapy was originally called IRS. This syndrome develops not only in patients with HIV infection but also in non-HIV immunocompetent hosts, such as patients with severe drug eruptions and those on immunosuppressive therapy, upon reduction or withdrawal of immunosuppressive agents or chemotherapy. Recently, IRS has also been reported to develop in lymphopenic and neutropenic patients [49] and patients receiving tumor necrosis factor (TNF) α inhibitors [55–58]. Clinical illness consistent with IRS includes tuberculosis, herpes zoster, herpes simplex, CMV infections, and sarcoidosis [53]. The manifestations of IRS are diverse and depend on the tissue burden of the preexisting infectious agents during the immunosuppressive state and the nature of the immune system being restored. Because in some cases IRS is self-limited within a week without any therapy while others are fatal or life-threatening, management of this syndrome should be decided on an individual basis (Table 1).

The clinical characteristics of IRS modified from criteria proposed by Shelburne et al. [59] are as follows: (1) paradoxical deterioration of preexisting infectious disease a attributable to the recovery of the immune system; (2) a decrease in the dose of pathogens, e.g., viral loads, with or without an increase in $CD4^+$ T cell counts; (3) clinical symptoms not explained by a newly acquired infection, by the expected clinical course of a previously recognized infectious agents, or by side effects of therapy; and (4) any event occurring after initiation of ART or after withdrawal or reduction of immunosuppressive agents including biologics, regardless of whether patients are HIV-positive or HIV-negative. In view of the observations that paradoxical worsening of clinical symptoms associated with reduction in viral loads is typically observed after withdrawal of the causative drug at onset of DiHS [15, 16, 35, 36], DiHS is likely a manifestation of the newly observed IRS [35, 51]. Various clinical observations in DiHS/DRESS could be explained by assuming that rapid restoration of pathogen-specific immunity after withdrawal of the causative drug with immunosuppressive properties, as described previously [15, 16, 53], would serve to reduce viral loads at onset, thereby rendering them undetectable in the blood. This consideration could explain why any herpesvirus DNA can be hardly detected at onset of DiHS/DRESS.

Systemic corticosteroids have been the mainstay of treatment for IRS and are the only treatment for which clinical trial data exist [53]. However, there have been no clear guidelines for how patients with IRS are treated with systemic corticosteroids. Because a mild form of IRS can respond to specific treatment for the underlying pathogens, immunosuppressive therapy is not generally needed, and the management is predominantly supportive. In patients with severe forms of IRS, however, immunosuppressive therapies in addition to antimicrobial therapies are necessary to ameliorate clinical symptoms [53]. In case of DiHS/DRESS, anti-microbial therapies should be avoided because they may increase the risk of

Table 1 Clinical illness consistent with IRS

Mycobacterium avium complex infection
Tuberculosis
Cryptococcosis
Herpes simplex
Herpes zoster
Hepatitis C virus infection
Hepatitis B virus infection
Cytomegalovirus infection
Sarcoidosis
Graves disease
Hashimoto thyroiditis
Drug-induced hypersensitivity syndrome

Modified from [53]

developing additional drug rashes due to cross-reactivity to multiple drugs, which has been reported to occur. During the course of DiHS/DRESS, systemic corticosteroids gave promising results in terms of ameliorating vigorous restoration of immune responses to pathogens, which is reflected in the clinical manifestations. Nevertheless, once systemic corticosteroids have started, drug dose should be reduced gradually upon resolution of clinical manifestation. We have to recognize that patients under immunosuppressive therapy, particularly those with DiHS/DRESS, are at greater risk of subsequently developing the wide spectrum of IRS ranging from herpes zoster to fatal CMV disease [41]. Our frequent monitoring of viral loads in the course of DiHS/DRESS revealed that the increase in CMV DNA loads coincided with a tapering of corticosteroid dose. This finding indicates that tapering corticosteroids more gradually over a prolonged period of time may help to limit the severity of IRS. The usual dose for the treatment of DiHS/DRESS is prednisolone 40–60 mg/kg. This dose needs to be tapered over 8–12 weeks to prevent the relapse of various symptoms as manifestation of IRS. The pattern of viral reactivations enhanced upon immune restoration would be different depending on the virus, immunosuppressive agents, or regimens. Given the ability of corticosteroids to reduce the EBV loads in patients with DiHS/DRESS, patients who are at risk of subsequently developing EBV-associated autoimmune disease may benefit from systemic corticosteroids.

Conclusion

Although we know that sequential reactivations of herpesviruses occur in many patients with DiHS/DRESS and that increased EBV loads during the course of the disease may lead to the generation of autoantibodies, the causal role of herpesviruses in the development of severe drug eruptions, if any, remains to be defined. Together with the current knowledge of anti-viral immune responses, we are now in a position to dissect the relative contribution of these responses to protective immunity and immunopathology.

Acknowledgments This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology (to T.S.) and the Health and Labour Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan (to T.S.).

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JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY

doi: 10.1111/1346-8138.12925

Journal of Dermatology 2015; **42**: 768–777

ORIGINAL ARTICLE

Efficacy of additional i.v. immunoglobulin to steroid therapy in Stevens–Johnson syndrome and toxic epidermal necrolysis

Michiko AIHARA,[1] Yoko KANO,[2] Hiroyuki FUJITA,[1,*] Takeshi KAMBARA,[3] Setsuko MATSUKURA,[3,†] Ichiro KATAYAMA,[4] Hiroaki AZUKIZAWA,[4] Yoshiki MIYACHI,[5] Yuichiro ENDO,[5] Hideo ASADA,[6] Fumi MIYAGAWA,[6] Eishin MORITA,[7] Sakae KANEKO,[7] Riichiro ABE,[8] Toyoko OCHIAI,[9] Hirohiko SUEKI,[10] Hideaki WATANABE,[10] Keisuke NAGAO,[11,‡] Yumi AOYAMA,[12] Koji SAYAMA,[13] Koji HASHIMOTO,[14] Tetsuo SHIOHARA,[2] for the SJS/TEN Study Group

[1]*Department of Dermatology, Yokohama City University School of Medicine, Yokohama,* [2]*Department of Dermatology, Kyorin University School of Medicine,* [9]*Department of Dermatology, Nihon University School of Medicine,* [10]*Department of Dermatology, Showa University School of Medicine,* [11]*Department of Dermatology, Keio University School of Medicine, Tokyo,* [3]*Department of Dermatology, Yokohama City University Medical Center, Yokohama,* [4]*Department of Dermatology, Osaka University Graduate School of Medicine, Osaka,* [5]*Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto,* [6]*Department of Dermatology, Nara Medical University, Kashihara,* [7]*Department of Dermatology, Shimane University Faculty of Medicine, Izumo,* [8]*Department of Dermatology, Hokkaido University School of Medicine, Sapporo,* [12]*Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama,* [13]*Department of Dermatology, Ehime University School of Medicine,* [14]*Ehime Prefectural University of Health Sciences, Ehime, Japan*

ABSTRACT

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare and life-threatening cutaneous adverse drug reactions. While there is no established therapy for SJS/TEN, systemic corticosteroids, plasma exchange and i.v. immunoglobulin (IVIG) have been used as treatment. The efficacy of IVIG is still controversial because total doses of IVIG used vary greatly from one study to another. The aim of this study was to evaluate the efficacy of IVIG, administrated for 5 days consecutively, in an open-label, multicenter, single-arm study in patients with SJS or TEN. IVIG (400 mg/kg per day) administrated for 5 days consecutively was performed as an additional therapy to systemic steroids in adult patients with SJS or TEN. Efficacy on day 7 of IVIG was evaluated. Parameters to assess clinical outcome were enanthema including ophthalmic and oral lesions, cutaneous lesions and general condition. These parameters were scored and recorded before and after IVIG. We enrolled five patients with SJS and three patients with TEN who did not respond sufficiently to systemic steroids before IVIG administration. All of the patients survived and the efficacy on day 7 of the IVIG was 87.5% (7/8 patients). Prompt amelioration was observed in skin lesions and enanthema in the patients in whom IVIG therapy was effective. Serious side-effects from the use of IVIG were not observed. IVIG (400 mg/kg per day) administrated for 5 days consecutively seems to be effective in patients with SJS or TEN. IVIG administrated together with steroids should be considered as a treatment modality for patients with refractory SJS/TEN. Further studies are needed to define the therapeutic efficacy of IVIG.

Key words: corticosteroid, i.v. immunoglobulin, Stevens–Johnson syndrome, therapy, toxic epidermal necrolysis.

Correspondence: Michiko Aihara, M.D., Ph.D., Department of Dermatology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Email: maihara1@med.yokohama-cu.ac.jp

*Present address: Novartis Pharma, Tokyo, Japan

†Present address: Shiga Medical Center for Adults, Moriyama, Japan

‡Present address: Dermatology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Other investigators in the SJS/TEN Study Group are listed in the Appendix 1.

Received 18 December 2014; accepted 25 March 2015.

INTRODUCTION

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are serious conditions characterized by necrosis of the skin and mucous membranes resulting in erythema, blisters/erosions and enanthema. SJS/TEN are usually caused by an allergic reaction to a drug, including antibiotics, non-steroidal anti-inflammatory agents, anticonvulsants and allopurinol.[1,2] Infection including mycoplasma infection is also known as a possible cause of SJS in young patients.[2–4] The mortality of SJS/TEN is quite high; the recent mortality rate is reported as 34% at 1 year for SJS/TEN in Europe[5] and 3% and 19% for SJS and TEN, respectively, in Japan.[6] Because TEN mostly occurs in patients with SJS, followed by rapid progression, it is now generally agreed that these two conditions are part of a spectrum of the same disease and only differ in the severity of the skin disorder.[7] Therefore, the clinical classification of patients is based on the epidermal detachment area; SJS involves detachment on less than 10% of the body surface area (BSA).[7] Although the exact pathomechanism of SJS/TEN remains unclear, keratinocyte death is thought to be caused by cytotoxic T cells, and triggered by soluble Fas ligand (FasL) or granulysin produced by activated T cells.[8–11]

Many therapeutic modalities, including systemic steroids, plasmapheresis and immunosuppressant drugs, have been utilized.[2,12–14] Despite many reports describing the efficacy of these modalities, none of them has been established as the standard in SJS/TEN care. Because naturally occurring Fas-blocking antibodies in IVIG are thought to inhibit Fas-mediated keratinocyte apoptosis,[10] i.v. immunoglobulin (IVIG) has been used as well,[15–17] but the efficacy of IVIG is still unresolved.[18–20] The seriousness and rarity of SJS/TEN make it difficult to assess the efficacy in a large clinical randomized controlled trial (RCT). Barron *et al.*[21] have conducted a meta-analysis with meta-regression of 13 observational studies including eight studies[16,22–28] on a controlled group in the period of 1966–2011 to assess IVIG in the treatment of SJS/TEN. They assessed the efficacy of IVIG based on the Severity-of-Illness Score for Toxic Epidermal Necrolysis (SCORTEN) scoring system[29] and showed that IVIG at doses of 2 g/kg or more appears to significantly decrease mortality.

We therefore conducted an open-label, multicenter, single-arm study to evaluate the efficacy of IVIG therapy at a dose of 2 g/kg in Japanese patients with SJS/TEN. The symptoms in these patients had progressed or were unchanged after systemic steroid therapy, so an additional treatment modality was needed.

Table 1. Japanese Ministry of Health, Labor and Welfare (JMHW) Diagnostic Criteria 2005 for SJS/TEN

Diagnostic criteria for Stevens–Johnson syndrome (SJS)
Clinical entity
SJS is a severe mucocutaneous disorder characterized by erythema, epidermal detachment (including blisters and erosions) and enanthema accompanied by high fever. SJS is mainly caused by a drug
Essential criteria (required)
Severe, hyperemic and/or hemorrhagic mucocutaneous lesions
Epidermal detachment involving less than 10% of the total body surface area
High-grade fever (≥38.0°C) in the absence of antipyretic therapy
Supportive findings
Flat atypical target lesions
Bilateral acute keratoconjunctivitis accompanied by ocular surface epithelial defect and/or pseudomembranous formation
Histological evidence of epidermal necrosis
Diagnosis
Fulfillment of all three essential criteria is necessary for definite diagnosis
Re-evaluation is necessary for final diagnosis due to the risk of progression to its more extreme variant type, toxic epidermal necrolysis (TEN)
Diagnostic criteria for toxic epidermal necrolysis (TEN)
Clinical entity
TEN is a severe mucocutaneous disorder characterized by extensive erythema, epidermal detachment (including blisters and erosions), and enanthema accompanied by high fever. The extent of epidermal detachment is more than 10% of the total body surface area. The cause of TEN is a drug in most patients
Essential criteria (required)
Epidermal detachment involving more than 10% of the total body surface area
Exclusion of staphylococcal scalded skin syndrome
High-grade fever (≥38.0°C) in the absence of antipyretic therapy
Supportive findings
Generalized macular or diffuse erythema
Enanthema including bilateral acute keratoconjunctivitis accompanied by ocular surface epithelial defect and/or pseudomembranous formation
Histological evidence of marked epidermal necrosis
Diagnosis
Fulfillment of all three essential criteria is necessary for definite diagnosis

METHODS

Patients

This study was conducted at 13 Japanese medical institutions that had dermatologists who were experienced in care of patients with severe drug eruption. The study population consisted of patients with a definitive diagnosis of SJS or TEN based on the Japanese diagnostic criteria (see Table 1). Patients who fulfilled the inclusion criteria and did not meet the exclusion criteria listed below were eligible for this study. Inclusion criteria were: (i) patients aged 20 years or older who gave written consent to participate in the study; and (ii) patients with progressing or unchanged symptoms after steroid therapy (≥20 mg/day of prednisolone equivalent) administrated for

2 days or more. To ensure the proper evaluation of IVIG efficacy and the safety of patients, exclusion criteria were patients who met any of the following criteria in order: (i) a SCORTEN of 4 or more (see Table 3; note that serum bicarbonate, one of the SCORTEN risk factors, was not determined because of concern about an increased burden on patients); (ii) concurrent drug-induced hypersensitivity syndrome; (iii) had received steroid pulse therapy or plasma exchange within 2 days or high-dose IVIG within 28 days, prior to the administration of IVIG; (iv) a history of shock or hypersensitivity to IVIG; (v) concurrent malignant tumors under treatment; (vi) concurrent multi-organ failure or serious respiratory disease; (vii) IgA deficiency, serious hepatic or renal (creatinine ≥2 mg/dL) impairment, serious cerebrovascular or cardiovascular disorder, and hemolytic or blood loss anemia; and (viii) a platelet count of 75 000/μL or less.

Study design

This was an open-label, multicenter, single-arm study started in October 2012 and finished in April 2014. The "Protocol" and "Consent Form and Information for Patients" were approved by the institutional review board of each study site prior to the study and performed in accordance with good clinical practice guidelines and the ethical principles of the Declaration of Helsinki. The observation of the first subject started in November 2012 and the follow up of the last subject was completed in April 2014.

Treatment

A dose of 400 mg/kg per day IVIG was administrated as an i.v. infusion for 5 days consecutively from day 1 to day 5 as an additional therapy to systemic steroids. Steroid dose was not changed from day −2 to day 7. The study drug was from a single batch manufactured by Nihon Pharmaceutical, Co., Ltd (Tokyo, Japan).

End-point

The primary efficacy end-point was the response rate as measured by the severity-of-illness score (Table 2) on day 7 of IVIG. The severity-of-illness score was set up for this study to evaluate the efficacy in early stages after the therapy. It is a rating scale that scores ophthalmic lesions, lip/oral lesions, cutaneous lesions and general condition, with a total score ranging 0–39. Based on the results of a retrospective survey using the severity-of-illness score, patients with a reduction of 6 or more from day 1 of the therapy were considered responders.

The secondary end-points were the changes over time in severity-of-illness score, extent of epidermal detachment and extent of erythema until day 20. Sequelae were monitored until discharge of patients.

To evaluate the safety of IVIG, patients were monitored for adverse events until day 20 of the therapy.

RESULTS

Patient characteristics and treatment before IVIG

The study comprised 41 patents with SJS/TEN who were admitted during the study period. Many of the patients recovered with steroid therapy and some patients had one or more exclusion criteria. Informed consent was obtained from nine patients. A patient whose symptoms resolved before IVIG was excluded from the study, so a total of eight patients were treated with IVIG. The diagnoses and symptoms of patients are shown in Table 4. Five patients had SJS (41–78 years old), and three patients had TEN (52–65 years old). All patients, except one who was suspected of having an infection, had been administrated drugs before symptoms of SJS/TEN appeared.)

The SCORTEN at the time of consent was one in all of the five patients with SJS, two in one patient with TEN, and three in two patients with TEN (average 1.63, the predictive number of deaths was 0.99).

The severity-of-illness score was 14 in two patients, 15 in two patients and 17 in one patient in the SJS group, and 22, 23 and 31, respectively, in three patients in the TEN group.

The IVIG was started from 3 to 23 days after the onset of cutaneous symptoms: 3 days (case 4), 7 days (case 8), 9 days (cases 3 and 6), 10 days (case 2), 12 days (case 1), 13 days (case 7) and 23 days (case 5) (median 10 days after onset of cutaneous symptoms).

Before starting IVIG, steroids were administrated at maximum doses of 20 mg/day (case 2), 30–60 mg/day (cases 1, 4, 6 and 8) of prednisolone or prednisolone equivalent, or as steroid pulse therapy, 1000 mg/day of methylprednisolone for 3 days; from day −6 to day −4 in cases 3 and 5, and from day −11 to day −9 in case 7. Case 2 was suspected to have undetermined infection; the steroid dose was not increased (see below).

All patients were treated with steroids for more than 2 days.

Efficacy results

Efficacy evaluation. The changes over time in severity-of-illness score are shown in Figure 1. The treatment history and changes over time in severity-of-illness score, extent of epidermal detachment and extent of erythema for each patient are shown in Figure 2.

The response rate as measured by the severity-of-illness score on day 7 of IVIG, the primary end-point of this study, was 87.5% (7/8 patients). One patient with TEN was considered a non-responder. In the seven responders, the mean severity-of-illness score was 17.1 (SJS, 14–17; TEN, 22–23) on day 1, then it started to decrease rapidly on day 4, and reached 4.1 (SJS, 1–6; TEN, 8 in all patients) on day 7, representing a mean score reduction of 13.0 from the score on day 1; on day 20, the symptoms had resolved almost completely. A patient with TEN (case 5) had a score of 28 on day 7, which was lower by 3 than the day 1 value of 31 but it failed to meet the response criteria (a reduction of ≥6), resulting in a non-responder assessment.

The extent of epidermal detachment including erosion and blister and that of erythema were also markedly reduced on day 7 of IVIG therapy in the seven patients who were considered responders according to their severity-of-illness score. In the seven responders, the mean extents of epidermal detachment and erythema were 9.4% (SJS, 0–9%; TEN, 18–30%)