ORIGINAL ARTICLE

The dynamics of herpesvirus reactivations during and after severe drug eruptions: their relation to the clinical phenotype and therapeutic outcome

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Abstract

Background: Drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (DIHS/DRESS) and Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) represent contrasting poles of severe drug eruptions, and sequential reactivations of several herpesviruses have exclusively been demonstrated in the former. No previous studies, however, were extended beyond the acute stage. We sought to investigate whether herpesvirus reactivations could also be observed in SJS/TEN and beyond the acute stage of both diseases.
Methods: Patients with SJS ($n = 16$), SJS/TEN overlap ($n = 2$), TEN ($n = 10$), and DIHS/DRESS ($n = 34$) were enrolled. We performed a retrospective analysis of Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), and cytomegalovirus (CMV) DNA loads sequentially determined by real-time polymerase chain reaction during a 2-year period after onset.
Results: Persistently increased EBV loads were detected in SJS during the acute stage and long after resolution, but not in others. In contrast, high HHV-6 loads were exclusively detected in DIHS/DRESS during the acute stage. The dynamics of herpesvirus reactivation varied in DIHS/DRESS according to the use of systemic corticosteroids: While EBV loads were higher in patients not receiving systemic corticosteroids, CMV and HHV-6 loads were higher in those receiving them.
Conclusions: Distinct patterns of herpesvirus reactivation according to the pathological phenotype and to the use of systemic corticosteroids were observed during the acute stage and follow-up period, which may contribute, at least in part, to the difference in the clinical manifestations and long-term outcomes. Systemic corticosteroids during the acute stage may improve the outcomes in DIHS/DRESS.

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are generally believed to be most severe adverse reactions to drug, characterized by the widespread destruction of the epithelium of the skin and mucous membranes (1, 2). Although the view that infectious agents caused SJS/TEN had seemed heretical 20 years ago, this view began to change 10 years ago, when some patients with SJS were found to be closely associated with *Mycoplasma pneumoniae* infection (3–5). Although a variety of infectious agents other than this organism, such as herpes simplex virus, have also been suggested to be linked to the development of SJS (6), it remains largely unknown whether

these organisms could be the primary driving force in the pathogenesis or play a secondary part, fostering disease only in hosts with pre-existing abnormalities of the immune response to drug. Given their common properties to induce massive expansions of cross-reactive memory T-cell populations (7, 8) and their persistence in the host (9), herpesviruses are the most likely additional factors involved in the pathogenesis of severe drug eruptions. Indeed, we for the first time reported the sequential occurrence of herpesvirus reactivations during the course of drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS), representing contrasting poles of

severe drug eruptions (10, 11). This event stimulated many investigators to systematically search for herpesvirus reactivation during the course of other severe drug eruptions, SJS/TEN. Although many previous studies performed real-time quantitative polymerase chain reaction (PCR) to detect and quantify viral DNA using blood sample sequentially obtained from patients after onset of rashes, most of these studies were performed with small samples and not extended beyond the acute stage of the disease. Thus, these previous studies are not sufficient to explain the difference in clinical manifestations and long-term outcomes associated with these severe drug eruptions, some of which occurred after a disease-free interval of several months to years (12–17). In this regard, more detailed longitudinal studies of patients may provide additional insights into the role of virus reactivations in the pathogenesis of severe drug eruptions and their long-term sequelae.

In this report, we describe detailed longitudinal studies of patients with severe drug eruptions over a follow-up period of 2 years. Our results suggest that the viral reactivation events associated with severe drug eruptions extend both beyond a simple ability to handle specific herpesvirus and beyond the time frame of the acute stage and that distinct patterns of herpesvirus reactivations observed in these patients may contribute, at least in part, to the marked difference in clinical manifestations and long-term outcome.

Materials and methods

Patients and real-time polymerase chain reaction

Patients with severe drug eruptions who visited our hospital between 1999 and 2012 were enrolled. This study has been approved by the Institutional Review Board at Kyorin University School of Medicine.

The severe adverse drug eruptions were divided into four groups according to the clinical presentation, SJS ($n = 16$), SJS/TEN overlap ($n = 2$), TEN ($n = 10$), and DIHS/DRESS ($n = 34$). Patients with eczema/dermatitis ($n = 17$) and anaphylactoid purpura ($n = 6$) were enrolled as control groups.

Diagnosis of SJS, TEN, and DIHS/DRESS was made based on their criteria (18, 19; Table 1). According to the criteria for differentiating erythema multiforme major (EMM) from SJS (20), we excluded cases with the suspicion of EMM. The causative drugs were withdrawn when the diagnosis of drug reactions was made.

All patients with SJS/TEN were treated with systemic corticosteroids 0.8–1 mg/kg daily. In contrast, 15 of 34 patients with DIHS/DRESS were treated with systemic corticosteroids 0.8–1 mg/kg daily after admission, while two of 34 patients were treated with systemic corticosteroids 0.2–0.3 mg/kg daily before the first presentation to our hospital, without dosage increments after administration. The others were treated with supportive therapy alone.

Table 1 Characteristics of patients

	SJS	SJS/TEN overlap	TEN	DIHS/DRESS
Age, years*	57.3 ± 21.0	36.0 ± 5.6	45.7 ± 19.9	57.9 ± 16.9
Gender (M/F)	11/5	0/2	2/8	20/14
Skin detachment†	4.1 ± 0.9	18.5 ± 6.5	50.0 ± 8.9	
SCORTEN scale‡	1.1 ± 0.2	1.0 ± 0.0	2.1 ± 0.4	
Underlying illness (no. of patients)	Brain tumor (1)	Psychological illness (1)	Arthropathy (1)	Brain tumor (1)
	Chronic renal failure (1)	Upper respiratory inflammation (1)	Basedow's disease (1)	Cerebrovascular disease (7)
	COPD (1)		Bronchial asthma (1)	Epilepsy (7)
	Epilepsy (1)		Cerebrovascular disease (1)	Fibromyalgia (1)
	Hyperuricemia (1)		Colon carcinoma (1)	Hyperuricemia (3)
	Mycoplasma infection (1)		Diabetes mellitus (1)	Postherpetic neuralgia (1)
	Pneumonia (1)		Hyperuricemia (1)	Psychological illness (6)
	Psychological illness (1)		Multiple sclerosis (1)	Rheumatoid arthritis (1)
	Rectum carcinoma (1)		Pneumonia (1)	Spondylopathy (1)
	Spondylopathy (1)		Spondylitis (1)	
	Upper respiratory inflammation (1)		Ulcerative colitis (1)	

The clinical criteria used for the diagnosis of Stevens–Johnson syndrome (SJS) were widespread erythematous macules or flat atypical targets and detachment below 10% of the body surface area; those for SJS/TEN overlap were widespread erythematous macules or flat atypical targets and detachment between 10% and 30% of the body surface area; those for TEN were widespread erythematous macules or flat atypical targets and detachment above 30% of the body surface area (18); and those for the drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (DIHS/DRESS) were high fever, a widespread maculopapular and/or diffuse erythematous eruption, lymphadenopathy, leukocytosis with atypical lymphocytosis and/or eosinophilia, liver dysfunction, and human herpesvirus 6 (HHV-6) reactivation (19).
*Mean age ± SD.
†Mean percentage ± SEM.
‡Number of risk factors ± SEM.

Blood samples were obtained at or near the time of the initial presentation before starting therapy and thereafter on a biweekly basis during the course of the disease until resolution. Additional samples were also sequentially obtained on a several monthly basis for 2 years after onset. Real-time Epstein–Barr virus (EBV), HHV-6, and cytomegalovirus (CMV) DNA PCR was performed, as previously described (15). Blood samples obtained at the various time points were classified into five stages depending on the timing of sampling: days 1–10, days 11–30, days 31–100, days 101–300, and day 301 onward after the onset.

Statistical analysis

Data were analyzed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Differences in age and number of virus DNA genome copies in 10^6 peripheral leukocytes between the groups were analyzed by Welch's t-tests, while differences in gender and rate of patients with increased virus DNA loads were analyzed by Fisher's exact tests. Significance was defined as P value of 0.05 or less for all tests.

Results

Detection of EBV DNA at onset in patients with SJS

We initially determined EBV, HHV-6, and CMV DNA loads at or near the time of the initial presentation. Increased EBV DNA loads defined as >200 genome copies/10^6 leukocytes were detected within 10 days after the onset of rash in half of patients with SJS examined (40% in cases before systemic corticosteroid therapy), but in <20% of patients with TEN. In contrast, <10% of patients with DIHS/DRESS had increased EBV DNA levels in blood samples, while only a few control patients had low levels of EBV DNA in their blood (SJS *vs* DIHS/DRESS, P < 0.05 Fisher's exact tests; Fig. 1). As shown in Fig. 2, the median concentration of EBV DNA in the blood from patients with SJS at the acute stage was much higher than that in those with DIHS/DRESS. Importantly, EBV DNA in two patients with SJS was detected as early as day 4 of skin rashes, much earlier than in those with DIHS/DRESS. The increase in EBV DNA loads in patients with SJS was not correlated with symptom severity, white blood cell count, or other immunological parameters at the acute stage.

Distinct patterns of herpesvirus reactivation according to the pathological phenotype during the acute stage and follow-up period

As shown in Fig. 3A, the mean EBV DNA loads were approximately one log higher in patients with SJS than in those with DIHS/DRESS during the acute stage and remained increased for a prolonged period after clinical resolution. In contrast, small numbers of patients with DIHS/DRESS and TEN had EBV load levels comparable to those with SJS only late during the course of the disease, usually on days 31–100 (Fig. 3A).
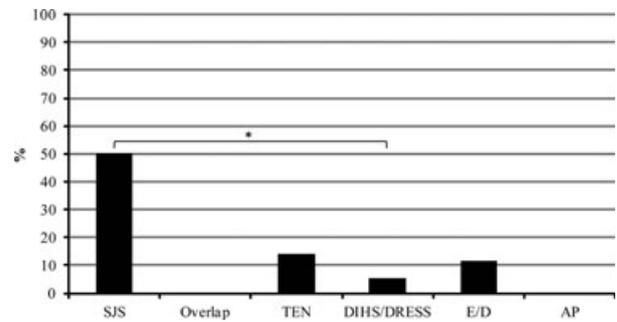

Figure 1 Frequencies of patients and controls with increased Epstein–Barr virus DNA loads, defined as more than 200 genome copies/10^6 leukocytes in their blood samples obtained within 10 days after the onset of rash. Abbreviations: AP, anaphylactoid purpura; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; E/D, eczema/dermatitis; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis. *P < 0.05 Fisher's exact tests.
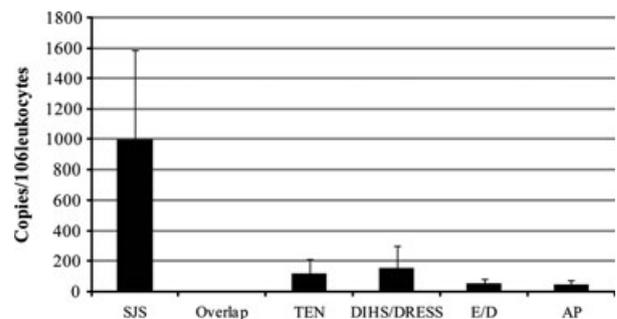

Figure 2 The mean values of Epstein–Barr virus DNA loads (genome copies/10^6 leukocytes) ± SEM in patients with severe drug eruptions and controls, whose blood samples were obtained within 10 days after the onset of rash. Abbreviations: AP, anaphylactoid purpura; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; E/D, eczema/dermatitis; SEM, standard error of the mean; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis. P = N.S. for all comparisons.

Cytomegalovirus reactivations occurred in 17.6% of patients with DIHS/DRESS and in 22.2% of those with SJS/TEN (Fig. 3B). During CMV reactivations, the patients showed a variety of clinical symptoms, including low-grade fever, rash, liver dysfunction, enterocolitis, hemorrhagic diarrhea, and pneumonia. In these patients with CMV reactivations, high levels of HHV-6 DNA were also detected prior to the detection of CMV DNA in the blood of patients with DIHS/DRESS. Patients who showed hemorrhagic diarrhea and enterocolitis were treated with ganciclovir.

Human herpesvirus 6 reactivations occurred in all patients with DIHS/DRESS at 2–4 weeks after onset, while no patients with SJS/TEN showed HHV-6 reactivations at any time point (Fig. 3C). During HHV-6 reactivations, the
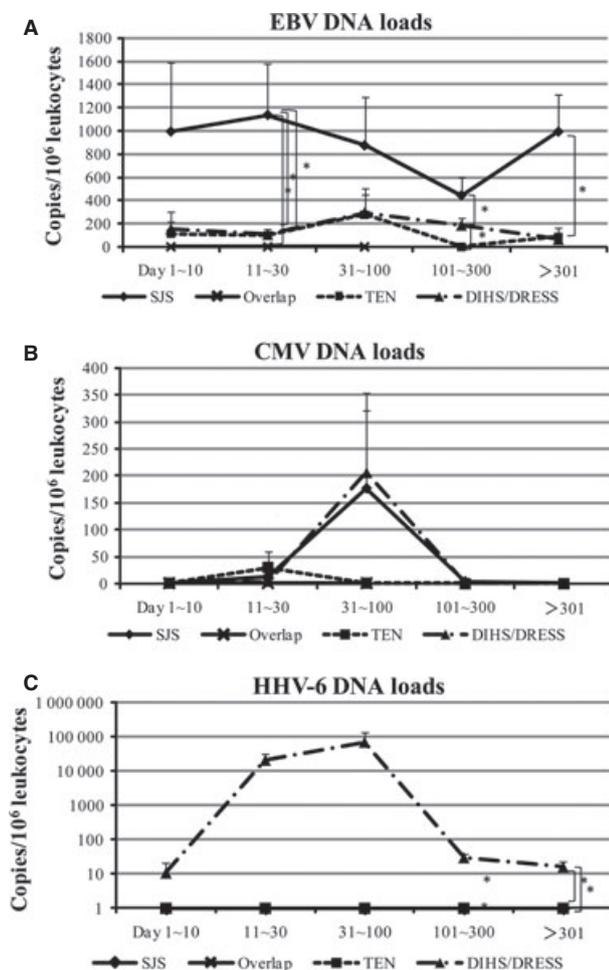
Figure 3 The mean values of Epstein–Barr virus (EBV; A), cytomegalovirus (CMV; B), and human herpesvirus 6 (HHV-6; C) DNA loads (genome copies/10^6 leukocytes) ± standard error of the mean (SEM) in patients with Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS) in the blood obtained at the various time points after onset are determined according to the timing of sampling as follows: days 1–10, days 11–30, days 31–100, days 101–300, and day > 301 after the onset. *$P < 0.05$ Welch's t-tests. Detailed data of this figure are shown in Table 2.

patients showed a variety of clinical symptoms, including low-grade fever, rash, and liver dysfunction; one patient developed limbic encephalitis.

The detailed data of Fig. 3 are shown in Table 2.

Effect of immunosuppressive drugs on virus reactivations

Because half of patients with DIHS/DRESS were treated with systemic corticosteroids and other half were not given any immunosuppressive agents, these patients were divided into two groups: steroid-treated and nontreated groups. These two groups were matched in age, and no statistical

difference was seen in the severity at their initial presentation to our hospital: They included fever, body surface area, leukocyte and eosinophil count, levels of serum alanine aminotransferase, IgG, and C-reactive protein. The changes in EBV, CMV, and HHV-6 viral loads before, during, and following therapy were monitored for 2 years after onset, to investigate the effect of corticosteroids on viral reactivations. As shown in Fig. 4, the difference in viral loads and the duration of viral reactivations between patients treated with and without corticosteroids was clear. The EBV DNA loads were apparently lower in the steroid-treated group than in the nontreated group. In contrast, the blood of the steroid-treated group had more CMV and HHV-6 DNA than did the blood from the nontreated group. The mean duration of CMV and HHV-6 reactivations was also longer in the steroid-treated group than in the nontreated group. Systemic corticosteroids appeared to exert the opposite effects on EBV and HHV-6/CMV DNA loads, although the limitations of this study are mostly a result of its retrospective aspect.

The outcomes of these patients were as follows. One patient with DIHS/DRESS died of CMV enterocolitis 3 months after onset of rashes (15): His blood sample had shown the highest CMV DNA load detected in this study. In this patient, fatal CMV enterocolitis developed when he was placed on a gradual reducing dose of prednisone, from 40 to 30 mg. Three patients with DIHS/DRESS died several months after onset due to other infections or other complications. One patient with SJS died of complications subsequent to the development of diffuse large B-cell lymphoma (DLBCL) 2 years after onset, in whom EBV DNA loads persistently increased after resolution (21).

Discussion

No longitudinal studies of patients with severe drug eruptions have been performed despite sporadic case reports describing severe long-term sequelae, which developed after a disease-free interval of several months to years; several studies reported that autoimmune disorders could occur as a sequela of DIHS/DRESS (13, 14, 16), while short- or long-term complications of SJS/TEN are persistent ocular changes, such as severe dry eyes, vision loss, and bronchiolitis obliterans (22–25). Thus, different complications could develop at various times after clinical resolutions of DIHS/DRESS and SJS/TEN, respectively. Longitudinal studies could help determine whether an increase in viral loads occurs in association with the development of severe drug eruptions or whether individuals with increased viral loads are at greater risk of developing severe drug eruptions. Our real-time PCR analysis revealed a significantly higher level of EBV DNA in the blood from patients with SJS at onset than that from patients with other severe drug eruptions and other skin diseases: In contrast, only a fraction of patients with DIHS/DRESS had EBV DNA identified at onset. In addition, the EBV viral load observed during remission, long after clinical resolution, in many patients with SJS was in the same range as what was observed in these patients during the acute stage of SJS. In many patients with SJS, EBV DNA, although even

Table 2 Detailed data of figure 3

	Days				
	1–10	11–30	31–100	101–300	301–
SJS					
EBV DNA					
Mean*	994.8	1137.4	874.0	444.1	993.8
SE†	591.7	441.2	416.4	158.8	324.7
Max‡	3500.0	4100.0	3300.0	1000.0	1500.0
Freq (over 20)§	66.7	90.0	70.0	85.7	100.0
Freq (over 200)¶	50.0	60.0	50.0	57.1	75.0
n**	6	10	10	7	4
Samples††	8	14	19	11	15
CMV DNA					
Mean*	0.0	13.8	176.8	4.0	0.0
SE†	0.0	9.5	145.3	4.0	0.0
Max‡	0.0	48.0	890.0	24.0	0.0
Freq (over 20)§	0.0	40.0	33.3	16.6	0.0
Freq (over 200)¶	0.0	0.0	16.7	0.0	0.0
n	3	5	6	6	1
Samples††	4	7	14	8	3
HHV-6 DNA					
Mean*	0.0	0.0	0.0	0.0	0.0
SE†	0.0	0.0	0.0	0.0	0.0
Max‡	0.0	0.0	0.0	0.0	0.0
Freq (over 20)§	0.0	0.0	0.0	0.0	0.0
Freq (over 200)¶	0.0	0.0	0.0	0.0	0.0
n**	6	5	5	1	3
Samples††	7	6	7	1	4
Overlap					
EBV DNA					
Mean*	0.0	0.0	0.0		
SE†	0.0	0.0	0.0		
Max‡	0.0	0.0	0.0		
Freq (over 20)§	0.0	0.0	0.0		
Freq (over 200)¶	0.0	0.0	0.0		
n**	2	2	2	0	0
Samples††	2	2	2	0	0
CMV DNA					
Mean*	0.0	0.0	0.0		
SE†	0.0	0.0	0.0		
Max‡	0.0	0.0	0.0		
Freq (over 20)§	0.0	0.0	0.0		
Freq (over 200)¶	0.0	0.0	0.0		
n**	2	2	2	0	0
Samples††	2	2	3	0	0
HHV-6 DNA					
Mean*	0.0	0.0	0.0		
SE†	0.0	0.0	0.0		
Max‡	0.0	0.0	0.0		
Freq (over 20)§	0.0	0.0	0.0		
Freq (over 200)¶	0.0	0.0	0.0		
n**	2	2	2	0	0
Samples††	2	2	2	0	0
TEN					
EBV DNA					
Mean*	114.3	98.5	276.6	0.0	85.6
SE†	98.6	54.5	223.4	0.0	74.2
Max‡	700.0	340.0	1800.0	0.0	380.0
Freq (over 20)§	28.5	50.0	25.0	0.0	40.0
Freq (over 200)¶	14.2	16.7	25.0	0.0	20.0

Table 2 (Continued)

	Days				
	1–10	11–30	31–100	101–300	301–
n**	7	6	8	3	5
Samples††	9	6	14	3	5
CMV DNA					
Mean*	0.0	30.3	0.0	0.0	0.0
SE†	0.0	29.9	0.0	0.0	0.0
Max‡	0.0	120.0	0.0	0.0	0.0
Freq (over 20)§	0.0	25.0	0.0	0.0	0.0
Freq (over 200)¶	0.0	0.0	0.0	0.0	0.0
n**	2	4	4	2	4
Samples††	3	5	12	2	4
HHV-6 DNA					
Mean*	0.0	0.0	0.0	0.0	0.0
SE†	0.0	0.0	0.0	0.0	0.0
Max‡	0.0	0.0	0.0	0.0	0.0
Freq (over 20)§	0.0	0.0	0.0	0.0	0.0
Freq (over 200)¶	0.0	0.0	0.0	0.0	0.0
n**	2	3	3	1	5
Samples††	3	4	6	1	5
DIHS/DRESS					
EBV DNA					
Mean*	154.5	106.0	296.0	186.2	66.7
SE†	144.0	43.8	152.9	67.4	35.9
Max‡	2600.0	920.0	3300.0	1200.0	330.0
Freq (over 20)§	21.4	38.0	50.0	57.9	33.3
Freq (over 200)¶	5.6	23.8	36.3	31.6	16.7
n**	18	26	22	19	12
Samples††	19	41	64	46	23
CMV DNA					
Mean*	0.0	4.6	205.7	0.0	0.0
SE†	0.0	2.5	147.2	0.0	0.0
Max‡	0.0	45.0	3400.0	0.0	0.0
Freq (over 20)§	0.0	12.0	26.1	0.0	0.0
Freq (over 200)¶	0.0	0.0	26.1	0.0	0.0
n**	10	25	23	15	7
Samples††	14	40	66	34	14
HHV-6 DNA					
Mean*	10.7	20897.8	69707.1	30.0	15.9
SE†	10.7	12418.9	69558.8	9.8	8.2
Max‡	160.0	300000.0	1600000.0	110.0	96.0
Freq (over 20)§	5.3	56.7	47.8	46.7	30.7
Freq (over 200)¶	0.0	26.7	21.7	0.0	0.0
n**	15	30	23	15	13
Samples††	21	55	67	38	24

CMV, cytomegalovirus; DIHS/DRESS, drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms; EBV, Epstein–Barr virus; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; HHV-6, human herpesvirus 6.

*Mean DNA load (copies/10^6 leukocytes).

†Standard error.

‡Maximum DNA load (copies/10^6 leukocytes).

§Rate of patients with positive DNA load determined over 20 copies/10^6 leukocytes.

¶Rate of patients with high DNA load determined over 200 copies/10^6 leukocytes.

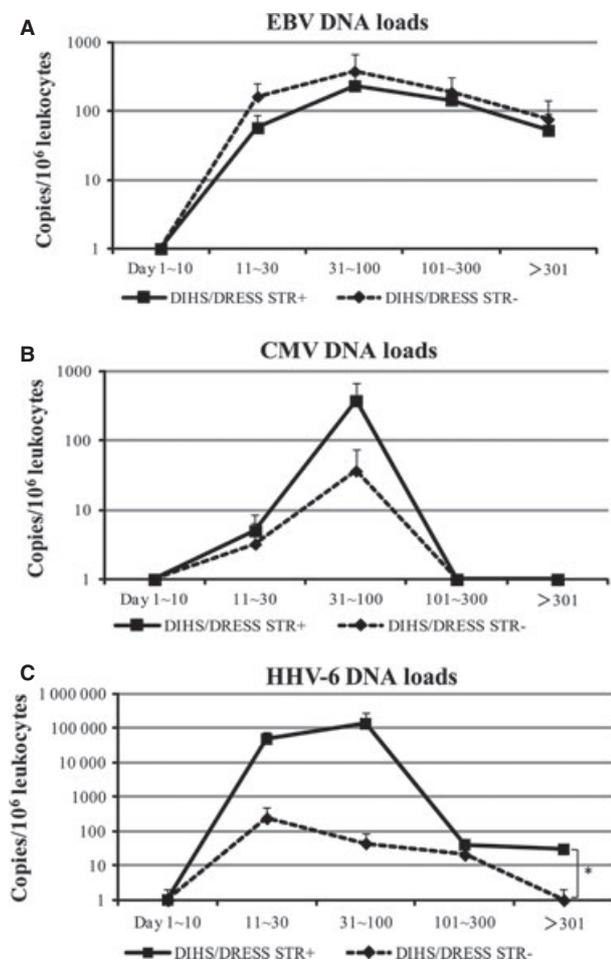**Number of studied patients.

††Number of studied samples.

Figure 4 The effect of systemic corticosteroids on Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus 6 (HHV-6) viral loads in patients with drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS). Patients with DIHS/DRESS are divided into two groups: patients treated with systemic corticosteroid (STR+; $n = 15$) and supportive therapy alone (STR-; $n = 17$). Only patients without any detectable viral DNA loads at their initial presentation were used for this analysis to avoid the criticism of selection bias that may have been associated with the patients with increased viral loads before treatment. The mean values of EBV (A), CMV (B), and HHV-6 (C) DNA loads (genome copies/10^6 leukocytes) ± standard error of the mean (SEM) of these groups are shown. *$P < 0.05$ Welch's t-tests.

in very low titers in some patients, persisted for up to 2 years after successful therapy. These results suggest that patients with high EBV DNA loads are at risk of subsequently developing SJS, although we could not totally exclude the alternative possibility that the aggressive clinical course observed during the acute phase of SJS may be responsible for EBV reactivations: However, this alternative possibility is unlikely because the degree of the EBV viral loads in patients with SJS did not correlate with the severity of clinical symptoms

and laboratory abnormalities during the acute stage (e.g., fever, body surface area, the SCORTEN score, and serum transaminase levels). Moreover, given the ability of corticosteroids to rather reduce the EBV viral loads in patients with DIHS/DRESS, EBV reactivation in patients with SJS is unlikely to be a direct consequence of corticosteroid therapy.

The higher EBV load specifically observed in patients with SJS may be caused by different factors. The most obvious explanation is that the observed increase in viral loads could reflect expansions of EBV-infected memory cells such as B cells. However, not consistent with this view, patients with SJS revealed a dramatic decrease in circulating T- and B-cell numbers at the acute stage (26). Alternatively, it is possible that the increase could be the result of destruction of EBV-specific $CD4^+$ and $CD8^+$ T cells during the active stage, thereby facilitating EBV persistence. This partly provides explanation for why some patients revealed higher EBV loads during the active stage. However, in view of persistence of high titers of EBV DNA observed even during remission in many patients with SJS, our finding could be interpreted as suggesting that SJS may develop in patients who are not capable of adequately mounting effective immune responses to the reactivating EBV.

Surprisingly, we noted that the vast majority of patients with TEN, except two, did not demonstrate increased EBV loads. 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 EBV reactivation, although they share important common pathophysiologic processes. If so, we could hypothesize that increased EBV loads at the acute stage of SJS may have served to prevent further progression to TEN. Nevertheless, the predictive value of EBV loads for SJS must be regarded cautiously because it was derived from small numbers of patients in our study. Thus, important limitation of the present study was the relatively small numbers of patients that were available for analysis and is mostly a result of its retrospective aspect. Some patients were also lost to follow up after the treatment.

The persistently increased EBV loads observed in patients with SJS may be one factor that predisposes to the subsequent development of EBV-associated lymphoproliferative disease (27, 28). Indeed, we have recently seen a patient with SJS who subsequently developed DLBCL 2 years after complete resolution of SJS (21): This patient revealed persistently increased EBV loads during which the patient had remained symptom free after the resolution of SJS, suggesting that this patient may have had defects in long-term anti-EBV immunity.

Because HHV-6 and CMV viral loads were higher in patients with DIHS/DRESS receiving corticosteroids compared with those without corticosteroid therapy, the degree and duration of HHV-6 and CMV reactivation would be greatly influenced by the use of immunosuppressive drugs. Systemic corticosteroids, however, did not enhance EBV reactivation, contrary to our initial prediction. These findings indicate that the pattern of viral reactivations enhanced by immunosuppressive agents or regimens would be different

according to the virus, agents, or regimens and suggest that patients receiving systemic corticosteroids are more likely to develop CMV disease and HHV-6-associated clinical symptoms. Thus, although patients with DIHS/DRESS are usually treated with corticosteroids, it remains to be determined which therapy can significantly reduce the degree and duration of virus reactivations and prevent long-term sequelae. In this regard, we have recently demonstrated that long-term sequelae such as autoimmune disease and autoantibody production were much more common in patients with DIHS/DRESS not treated with corticosteroids than in those treated with corticosteroids (29). These findings may indicate that systemic corticosteroid therapy during the acute stage may have served to reduce the risk of subsequently developing autoimmune disease through the beneficial effect of corticosteroids on EBV loads demonstrated here.

In conclusion, our findings justify the frequent monitoring of herpesvirus reactivation, particularly EBV reactivation, in patients with severe drug eruptions, to predict and improve the short-term or long-term outcome, and our findings should be further explored for their variability and validity in a study with a larger sample size.

Acknowledgments

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. *N Engl J Med* 1994;**331**:1272–1285.

2. Pereira FA, Mudgil AV, Rosmarin DM. Toxic epidermal necrolysis. *J Am Acad Dermatol* 2007;**56**:181–200.

3. Tay YK, Huff JC, Weston WL. *Mycoplasma pneumoniae* infection is associated with Stevens–Johnson syndrome, not erythema multiforme (von Hebra). *J Am Acad Dermatol* 1996;**35**:757–760.

4. Schalock PC, Dinulos JG. *Mycoplasma pneumoniae*-induced Stevens–Johnson syndrome without skin lesions: fact or fiction? *J Am Acad Dermatol* 2005;**52**:312–315.

5. Ravin KA, Rappaport LD, Zuckerbraun NS, Wadowsky RM, Wald ER, Michaels MM. *Mycoplasma pneumoniae* and atypical Stevens–Johnson syndrome: a case series. *Pediatrics* 2007;**119**:1002–1005.

6. Forman R, Koren G, Shear NH. Erythema multiforme, Stevens–Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. *Drug Saf* 2002;**25**:965–972.

7. Burrows SR, Silins SL, Moss DJ, Khanna R, Misko IS, Argaet VP. T cell receptor repertoire for a viral epitope in humans is diversified by tolerance to a background major histocompatibility complex antigen. *J Exp Med* 1995;**182**:1703–1715.

8. Rist M, Smith C, Bell MJ, Burrows SR, Khanna R. Cross-recognition of HLA DR4 alloantigen by virus-specific CD8+ T cells: a new paradigm for self-/nonself-recognition. *Blood* 2009;**114**:2244–2253.

9. Williams H, Crawford DH. Epstein–Barr virus: the impact of scientific advances on clinical practice. *Blood* 2006;**107**: 862–869.

10. Suzuki Y, Inagi R, Aono T, Yamanishi K, Shiohara T. Human herpesvirus 6 infection as a risk factor for the development of severe drug-induced hypersensitivity syndrome. *Arch Dermatol* 1998;**134**:1108–1112.

11. Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol* 2007;**33**:124–133.

12. Kamada N, Kinoshita K, Togawa Y, Kobayashi T, Matsubara H, Kohno M et al. Chronic pulmonary complications associated with toxic epidermal necrolysis: report of a severe case with anti-Ro/SS-A and a review of the published work. *J Dermatol* 2006;**33**:616–622.

13. Chiou CC, Yang LC, Hung SI, Chang YC, Kuo TT, Ho HC et al. Clinicopathological features and prognosis of drug rash with eosinophilia and systemic symptoms: a study of 30 cases in Taiwan. *J Eur Acad Dermatol Venereol* 2008;**22**:1044–1049.

14. Aota N, Shiohara T. Viral connection between drug rashes and autoimmune diseases: how autoimmune responses are generated after resolution of drug rashes. *Autoimmun Rev* 2009;**8**:488–494.

15. Asano Y, Kagawa H, Kano Y, Shiohara T. Cytomegalovirus disease during severe drug eruptions: report of 2 cases and retrospective study of 18 patients with drug-induced hypersensitivity syndrome. *Arch Dermatol* 2009;**145**:1030–1036.

16. Brown RJ, Rother KI, Artman H, Mercurio MG, Wang R, Looney RJ et al. Minocycline-induced drug hypersensitivity syndrome followed by multiple autoimmune sequelae. *Arch Dermatol* 2009;**145**:63–66.

17. Eshki M, Allanore L, Musette P, Milpied B, Grange A, Guillaume JC et al. Twelve-year analysis of severe cases of drug reaction with eosinophilia and systemic symptoms: a cause of unpredictable multiorgan failure. *Arch Dermatol* 2009;**145**:67–72.

18. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch Dermatol* 1993;**129**:92–96.

19. Shiohara T, Iijima M, Ikezawa Z, Hashimoto K. The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. *Br J Dermatol* 2007;**156**:1083–1084.

20. Côté B, Wechsler J, Bastuji-Garin S, Assier H, Revuz J, Roujeau JC. Clinicopathologic correlation in erythema multiforme and Stevens–Johnson syndrome. *Arch Dermatol* 1995;**131**:1268–1272.

21. Hirahara K, Kano Y, Ishida T, Shiohara T. Diffuse large B-cell lymphoma as a sequela of Stevens–Johnson syndrome associated with an increased Epstein–Barr virus load. *Eur J Dermatol* 2012;**22**:144–145.

22. Yip LW, Thong BY, Lim J, Tan AW, Wong HB, Handa S et al. Ocular manifestations and complications of Stevens–Johnson syndrome and toxic epidermal necrolysis: an Asian series. *Allergy* 2007;**62**:527–531.

23. Gregory DG. The ophthalmologic management of acute Stevens–Johnson syndrome. *Ocul Surf* 2008;**6**:87–95.

24. Mockenhaupt M. The current understanding of Stevens–Johnson syndrome and toxic epidermal necrolysis. *Expert Rev Clin Immunol* 2011;**7**:803–813.

25. Sugino K, Hebisawa A, Uekusa T, Hatanaka K, Abe H, Homma S. Bronchiolitis obliterans associated with Stevens–Johnson Syndrome: histopathological bronchial reconstruction of the whole lung and

immunohistochemical study. *Diagn Pathol* 2013;**8**:134.

26. Hirahara K, Kano Y, Mitsuyama Y, Takahashi R, Kimishima M, Shiohara T. Differences in immunological alterations and underlying viral infections in two well-defined severe drug eruptions. *Clin Exp Dermatol* 2010;**35**:863–868.

27. Rickinson AB, Kieff E. Epstein–Barr virus. In: Knipe DM, Howley PM, editors. *Fields Virology*, 4th edn. Philadelphia, PA: Raven Press; 2001: 2575–2627.

28. Young LS, Murray PG. Epstein–Barr virus and oncogenesis: from latent genes to tumours. *Oncogene* 2003;**22**:5108–5121.

29. Ushigome Y, Kano Y, Ishida T, Hirahara K, Shiohara T. Short- and long-term outcomes of 34 patients with drug-induced hypersensitivity syndrome in a single institution. *J Am Acad Dermatol* 2013;**68**:721–728.