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

Tetsuo Shiohara · Yukiko Ushigome · Yoko Kano · Ryo Takahashi

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

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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-$\alpha\beta$, 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

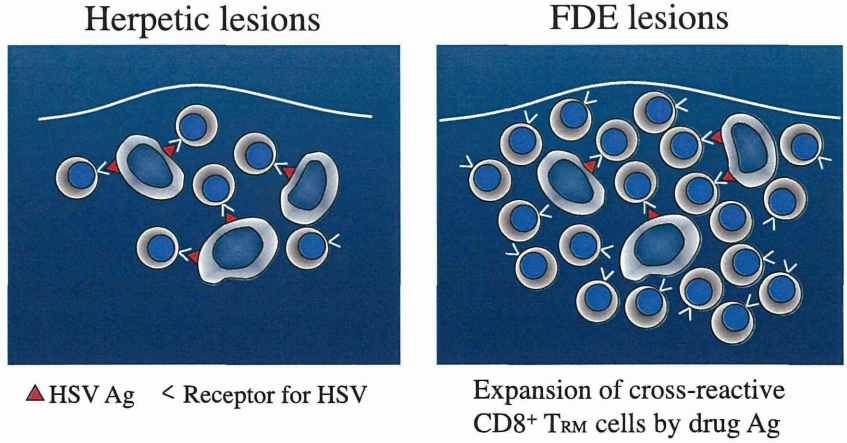

Fig. 1 HSV lesions evolve into FDE lesions. After infection with HSV, a small fraction of $CD8^+$ TRM cells specific for HSV persist as a stable population with antigen-presenting cells at the skin site of HSV infection to confer protection against the same virus. These T cells, once activated with cross-reactive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

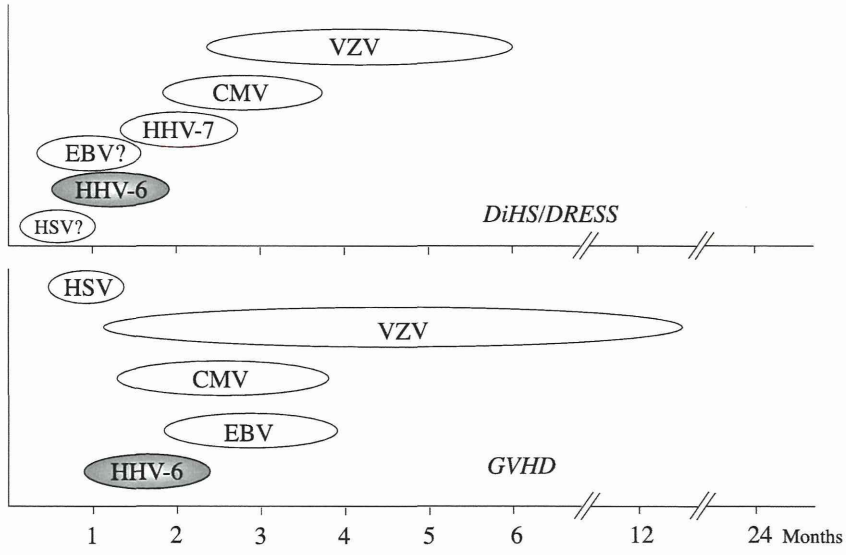

Fig. 2 Sequential reactivation of various herpesviruses during the courses of DiHS and GVHD. Importantly, herpesviruses are reactivated in DiHS in the fundamentally same order as in GVHD

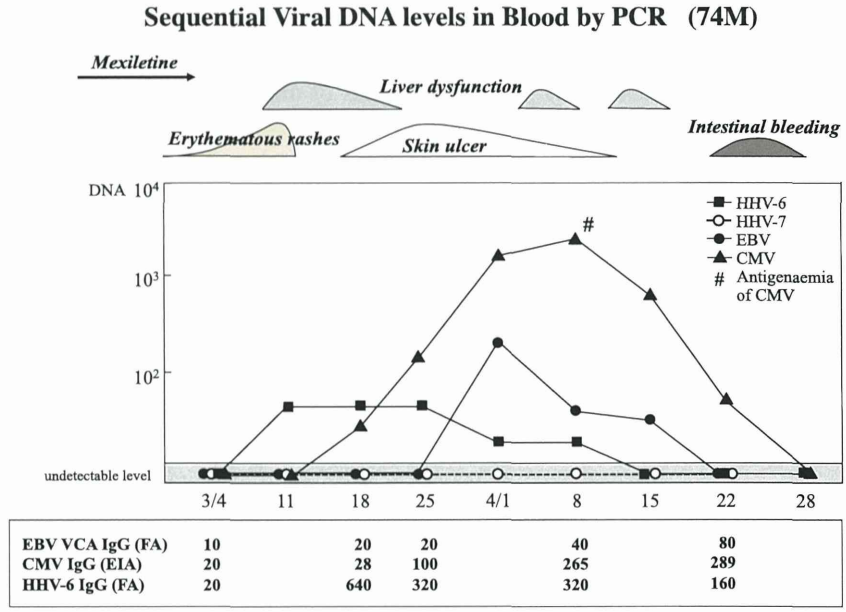

Fig. 3 Relation between clinical symptoms and herpesvirus viral DNA loads and anti-viral antibody titers in a representative case with DiHS due to phenobarbital

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-

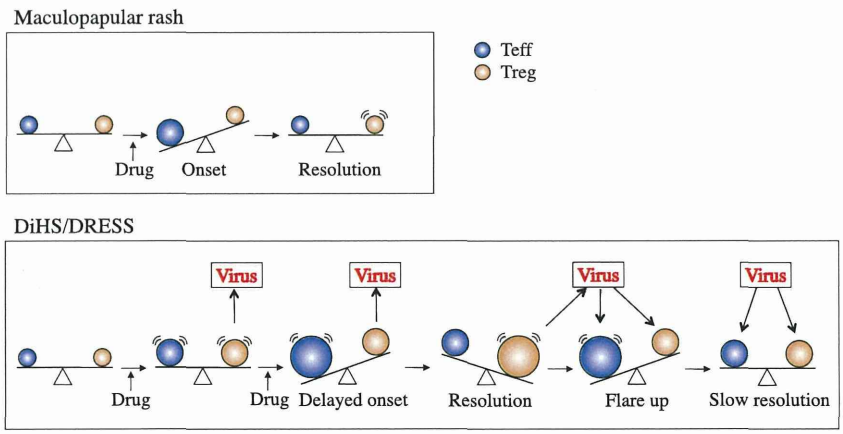

Fig. 4 A hypothetical model for the development of DiHS and maculopapular type of drug eruption. In maculopapular rash, Treg cells are overwhelmed with expansions of Teff cells. In DiHS, protracted use of the causative drug results in expansions of not only Teff but also Treg cells, thereby delaying 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

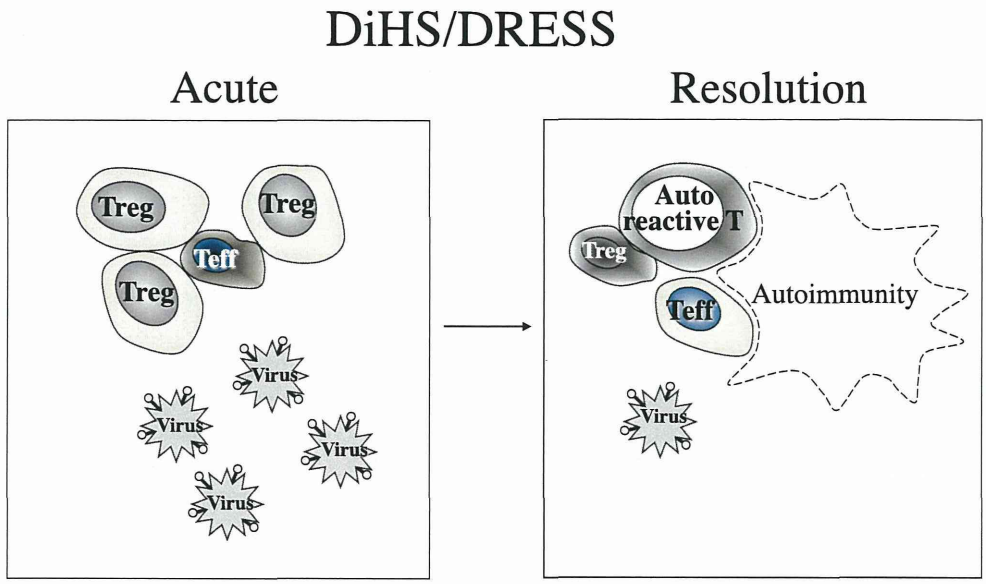

Fig. 5 Acute and resolution stages of DiHS. After resolution of clinical symptoms, Treg cells expanded during the acute stage are contracted and become exhausted in function, thereby triggering autoimmune diseases

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 anti-microbial 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.

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

T. Ishida, Y. Kano, Y. Mizukawa & T. Shiohara

Department of Dermatology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan

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Correspondence
Tadashi Ishida, Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka, Tokyo, Japan.
Tel.: +81-422-47-5511
Fax: +81-422-47-9632
E-mail: tadashi-i1584@ks.kyorin-u.ac.jp

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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) Chronic renal failure (1) COPD (1) Epilepsy (1) Hyperuricemia (1) Mycoplasma infection (1) Pneumonia (1) Psychological illness (1) Rectum carcinoma (1) Spondylopathy (1) Upper respiratory inflammation (1)	Psychological illness (1) Upper respiratory inflammation (1)	Arthropathy (1) Basedow's disease (1) Bronchial asthma (1) Cerebrovascular disease (1) Colon carcinoma (1) Diabetes mellitus (1) Hyperuricemia (1) Multiple sclerosis (1) Pneumonia (1) Spondylitis (1) Ulcerative colitis (1)	Brain tumor (1) Cerebrovascular disease (7) Epilepsy (7) Fibromyalgia (1) Hyperuricemia (3) Postherpetic neuralgia (1) Psychological illness (6) Rheumatoid arthritis (1) Spondylopathy (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).

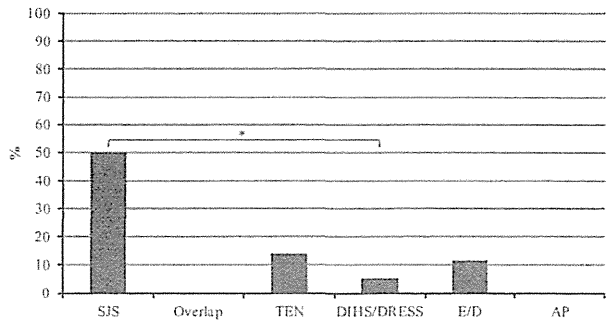

Figure 1 Frequencies of patients and controls with increased Epstein–Barr virus DNA loads, defined as more than 200 genome copies/10^6 leukocytes in their blood samples obtained within 10 days after the onset of rash. Abbreviations: AP, anaphylactoid purpura; DIHS, drug-induced hypersensitivity syndrome; 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.

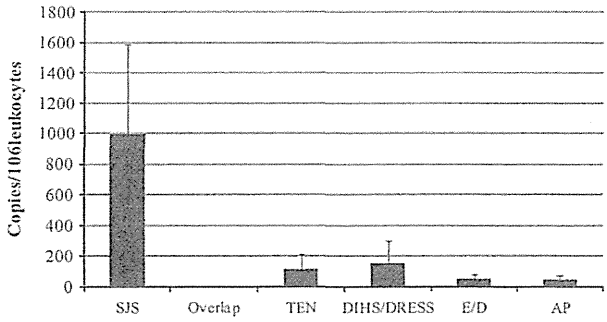

Figure 2 The mean values of Epstein–Barr virus DNA loads (genome copies/10^6 leukocytes) ± SEM in patients with severe drug eruptions and controls, whose blood samples were obtained within 10 days after the onset of rash. Abbreviations: AP, anaphylactoid purpura; DIHS, drug-induced hypersensitivity syndrome; 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

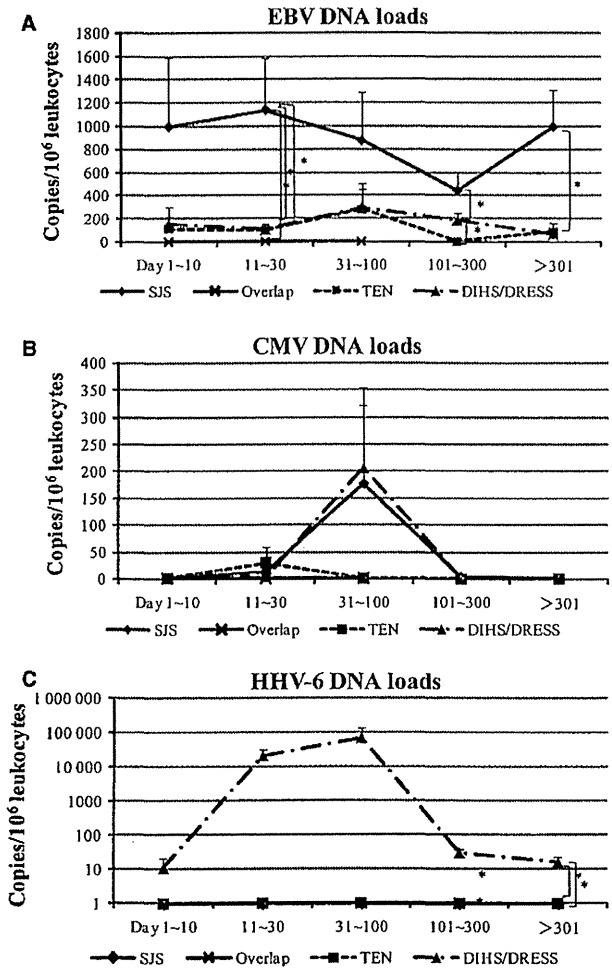

Figure 3 The mean values of Epstein–Barr virus (EBV; A), cytomegalovirus (CMV; B), and human herpesvirus 6 (HHV-6; C) DNA loads (genome copies/10^6 leukocytes) ± standard error of the mean (SEM) in patients with Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity 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
*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.

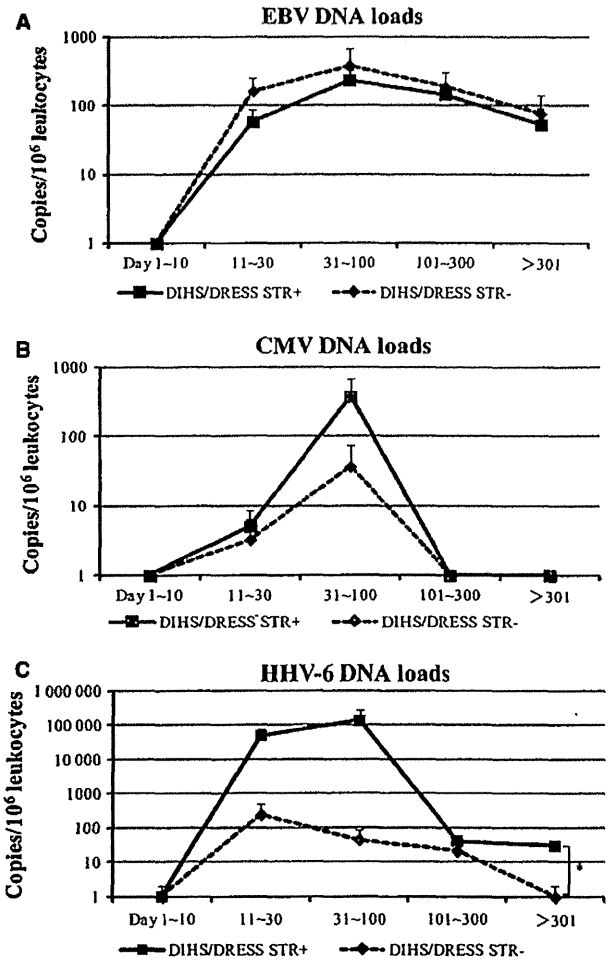

Figure 4 The effect of systemic corticosteroids on Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus 6 (HHV-6) viral loads in patients with drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS). Patients with DIHS/DRESS are divided 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

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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LETTER TO THE EDITOR

Pressure sore-like ulcers on acneiform papules caused by EGFR inhibitors

Dear Editors,

Epidermal growth factor receptor (EGFR) inhibitors such as erlotinib and gefitinib have been established as effective therapies for non-small cell lung cancer, pancreatic cancer, colorectal cancer, and head and neck cancer (1). However, a number of skin manifestations, such as acneiform papules, xerosis, photosensitive dermatitis, pruritus, perionychia and vasculitis have been reported in patients receiving EGFR inhibitors (2). Herein, we describe two Japanese patients who developed pressure sore-like ulcers on acneiform papules due to EGFR inhibitor treatment. Such ulcers have not been reported in the literature before.

Case 1: A 71-year-old woman suffering from metastatic and recurrent non-small cell lung cancer developed follicular papules or pustules on her face, trunk, buttocks and extremities after receiving gefitinib for 3 months. Furthermore, pressure sore-like ulcers with yellow necrotic tissue appeared on the sacral area (Figure 1A), the ischial area and the trochanter major area (Figure 2B). These ulcers were composed of multiple ulcerated papules or pustules. Clobetasol propionate was applied topically to the lesions and the ulcers, and the administration of gefitinib was then discontinued. The acneiform papules cleared within 10 days, and epithelialization of the ulcers occurred within 3 weeks.

Case 2: A 74-year-old man suffering from non-small cell lung cancer developed multiple follicular papules or pustules on the face, hypogastrium, inguen, ischial area and posterior aspect of the thigh after receiving erlotinib for 2 weeks. The pustules under pressure or shear (ischial area and inguen) were ulcerated and resembled a pressure sore. The posterior aspect of the thigh was subjected to pressure when the patient sat on a chair. Betamethasone butyrate propionate was administered topically to the lesions, including the ulcers. Moreover, the patient was advised to use a cushion to reduce the shearing and pressure. The lesions started to improve within few days. Finally, the papules and pustules cleared (Figure 2A), and epithelialization of the ulcers occurred within 3 weeks (Figure 2B).

EGFR is overexpressed in many solid cancers and is often associated with cancer development, growth, proliferation, metastasis and angiogenesis (3). It is also expressed in a wide variety of normal tissues, including epidermis, hair follicles and sebaceous glands. As a result, the skin is the organ that is most frequently affected by toxicity due to anti-EGFR therapy. Acneiform follicular papules or pustules without comedones or *Propionibacterium acnes* develop on the face, anterior upper chest and scalp in more than 90% of patients (2).

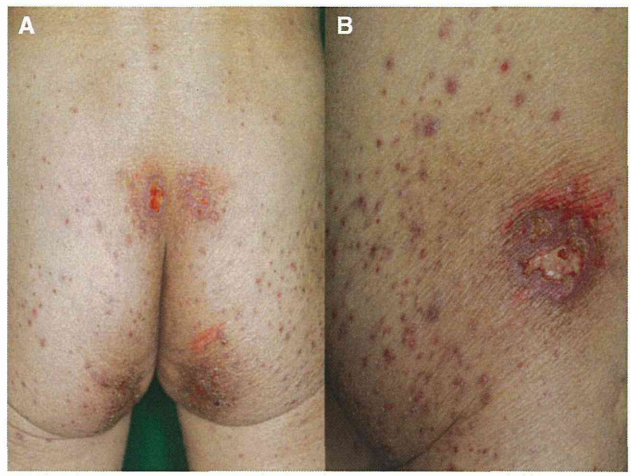

Figure 1 Case 1: Acneiform papules and pressure sore-like ulcers on the sacral area (A) and trochanter major area (B) after treatment with gefitinib for 3 months.

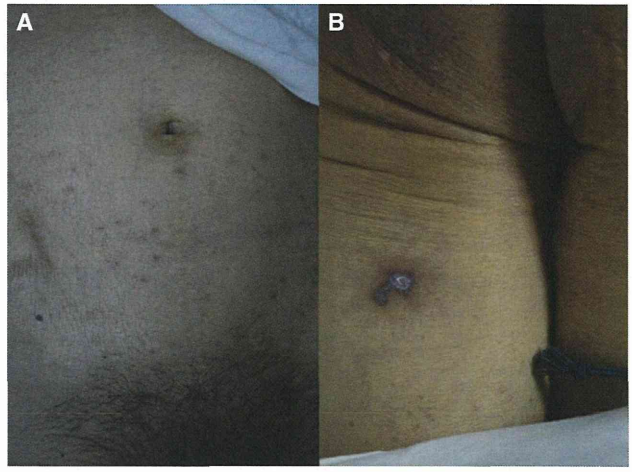

Figure 2 Case 2: Acneiform papules on the abdomen (A) and pressure sore-like ulcers on the posterior aspect of the thigh (B) after topical steroid therapy.

The National Pressure Ulcer Advisory Panel (NPUAP) defines a pressure ulcer as 'localized injury to the skin and underlying tissue, usually over a bony prominence, as a result of pressure in combination with shear.' Decreased physical activity, low-nutrient conditions, and friction and shear are well-known risk factors for pressure ulcers. Our two cases did not involve the risk factors described above.