bacterial and viral infections. An alternative hypothesis would be that the primary abnormality lies in the innate immune system that collectively affects the development and severity of AD [4]. In view of the clinical finding that there is no predisposition to any systemic infections in the majority of AD patients, we can postulate that there must be some intrinsic defects in innate defense mechanisms, particularly in the skin. Because regulatory T (T_{reg}) cells are shown to protect against the development of virus-induced inflammatory lesions [5], an imbalance between antiviral immune responses and T_{reg} cells is an alternative attractive candidate responsible for an increased susceptibility to certain viral infections in AD patients. In this review, we discuss recent advances in understanding viral infections associated with AD, with particular emphasis on T_{reg} cells and innate immune responses.

Clinical Aspects of Viral Complications in Atopic Dermatitis

AD patients run a higher risk of developing severe skin superinfections with a number of viruses including herpes simplex virus (HSV), molluscum contagiosum virus and vaccinia virus: after the causative virus they are named eczema herpeticum (EH), eczema molluscatum and eczema vaccinatum, respectively. EH caused by an extensive disseminated cutaneous infection with HSV 1 or 2 is the most commonly recognized viral complication in AD patients [6, 7]. EH was reported for the first time by Kaposi, who recognized similarities of this disease with varicella zoster virus (VZV) infection of the skin. Although the term 'Kaposi's varicelliform eruption' has still been widely used for complications of other skin diseases caused by a disseminated HSV infection, the term EH should be restricted to a disseminated HSV infection in AD or other types of dermatitis [7, 8]. EH can present in a primary form or a recurrent form, and the primary infection is generally considered to be severer with greater cutaneous involvement, lymphadenopathy and fever [9]. However, patients with recurrent HSV infections often develop disseminated vesicular lesions accompanied by systemic symptoms, such as fever, malaise and lymphadenopathy, findings indistinguishable from the primary infection. The eruption is most frequently located on the face, neck and the upper part of the chest, forearms and wrists while milder cases have lesions limited to the head and neck [9]. The vesicles rapidly evolve to pustules (fig. 1) or dry out, forming crusts. The vesiculopustular lesions tend to occur in areas where the skin has been most severely affected by the underlying skin disease in the primary form. Primary infections are thought to directly spread to a diseased cutaneous region by dissemination or autoinoculation. However, given that even recurrent EH lesions often occur in the previously affected site, the dissemination of vesiculopustular lesions is likely not true autoinoculation derived from the original infection site but may represent reactivation from viral latency at the site. Thus, it is difficult to distinguish between a primary infection and reactivation on clinical grounds alone without the aid of serological assays. These findings, together with the observation

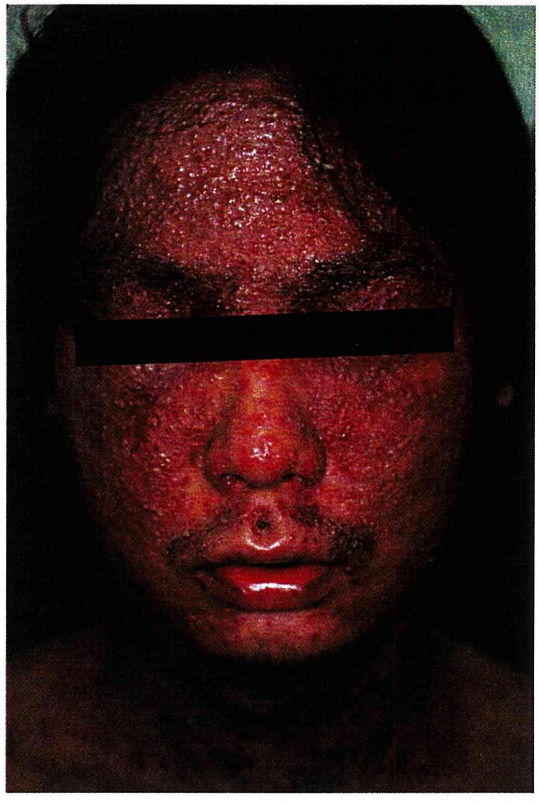

Fig. 1. Numerous, umbilicated pustules, erosions with purulent exudate and crusts cover the face and neck.

that severe, untreated AD lesions have EH develop more easily than patients with well-controlled disease [7], suggest that AD patients with uncontrolled eczematous lesions are at greater risk for the development of this viral complication. Consistently with this view, Wollenberg et al. [7] reported that the majority of the patients with EH had not received any corticosteroid therapy in the 4 weeks before the onset of EH, which suggests the possibility that EH may occur as a result of rebounding immune responses after withdrawal of topical corticosteroids. According to a retrospective study by Wollenberg et al. [7], risk factors for the development of EH are an early onset of AD and a high total serum IgE.

Although herpetic keratitis and herpetic meningitis are serious sequelae of EH, they are rare in the setting of EH [10]. Secondary bacterial infection is common and oral antibiotics are frequently given to treat bacterial superinfection. EH can often be associated with streptococcal impetigo, as a sequela of EH. Before the availability of acyclovir, the mortality rate of EH was approximately 75% [6]. The mainstay of therapy for EH is a nucleoside analog, such as acyclovir, valacyclovir and famciclovir. The use of topical or systemic corticosteroids is controversial, particularly in the acute phase of EH; however, most physicians suggest to avoid topical and systemic corticosteroids. Oral antibiotics are frequently given to prevent bacterial superinfection, because EH may be followed by group A β-hemolytic streptococcus or *Staphylococcus*

aureus infections. If no response is seen upon these therapies, resistance to acyclovir or related drugs is a possibility: these patients should be treated with foscarnet, vidarabine or cidofovir [11]. Because severe recurrence of EH can take place in some AD patients who often interrupt topical treatment with corticosteroids, prophylactic acyclovir or valacyclovir can be a therapeutic option that may reduce recurrence.

Pathomechanisms of Eczema Herpeticum

EH occurs almost exclusively in patients with AD, particularly in those who fail to control the skin lesions. The reason why patients with AD or those with a history of AD are at great risk for the development of EH remains unknown. A dysfunctional epidermal barrier and decreased innate immune responses are possible predisposing factors, both of which will be described in detail in the next section. Another mechanism whereby this may exclusively occur in patients with AD is the involvement of CD4+ T_{reg} cells. The protective immunity to HSV in humans depends on CD4+ and CD8+ T effector (T_{eff}) cell populations that recognize viral antigens: on the other hand, damage to the skin is also initiated by these T_{eff} cell populations [12, 13], pointing to a critical role of HSV-specific T cells not only in the resolution, but also in the initiation of HSV lesions. Because T_{reg} cells are known to suppress such excessive T_{eff} cell responses to HSV, thereby limiting T_{eff}-mediated immunopathology, it has been tempting to speculate that an imbalance in the proportion of T_{eff} to T_{reg} cells may contribute to the development of EH. There are conflicting data with respect to T_{reg} cells in AD: many studies have reported an increase in the frequencies of circulating T_{reg} cells in patients with AD [14–16], while others demonstrated that AD patients with active lesions had a lower T_{reg} cell level than asymptomatic control subjects with similar levels of serum γ-interferon, total IgE and eosinophils and that T_{reg} cell frequencies correlated inversely with the severity of disease [17, 18]. However, no previous studies assessed alterations in their frequencies and functional properties depending on the clinical symptoms in patients with AD, particularly those associated with EH. Thus, it remains unknown how alterations of T_{reg} cell frequencies and functions could contribute to the initiation and resolution of EH lesions. Nevertheless, T_{reg} cells in AD with recurrent episodes of EH have not been extensively studied during the course of the illness.

Accordingly, we sequentially analyzed T_{reg} cell numbers and function at different stages of the illness. Nine patients with adult-type AD, who developed EH, were enrolled in this study. Eight patients out of the 9 were positive for HSV IgG and had recurrent HSV infection but with or without a history of EH: they were defined as recurrent EH. One patient was positive for HSV IgM without HSV IgG and had no history of EH: the patient was assumed to have primary HSV infection. AD patients who were positive for HSV IgG but had no EH at their presentation were selected as age-matched AD controls and defined as ADEH–. AD patients with EH had a

significantly higher frequency of CD4+Foxp3+ T_{reg} cells in peripheral blood mononuclear cells at onset as compared with that after resolution and that in ADEH– cases. The increased T_{reg} cells at onset of EH exhibited phenotype characteristics similar to those in ADEH– patients and in healthy controls. After resolution of EH, the frequencies of Foxp3+ T_{reg} cells decreased to values similar to those in ADEH– and in healthy controls. These results suggest that expanded T_{reg} cells were contracted upon clinical resolution and that EH can be only aborted by a timely decrease in T_{reg} cell frequencies. We next analyzed T_{reg} cell frequencies in a single AD patient with primary EH over the ensuing 6-month period. In this patient, T_{reg} cells also increased at onset in the same range as what we observed in other AD patients with recurrent EH. Contrary to our expectation, however, after clinical resolution the frequency of T_{reg} cells remained elevated: the clinical improvement of EH was not associated with the decrease in T_{reg} cell frequency, a finding never observed in recurrent EH. However, the frequencies of T_{reg} cells eventually decreased. Since then, a flare of EH was no longer seen. These results indicate that a timely decrease in T_{reg} cell frequency may help prevent the further development of EH.

Because our recent study has demonstrated that the functional activity of T_{reg} cells was profoundly altered depending on the stage in severe drug eruptions [19], we next asked whether changes in T_{reg} cell frequency could be associated with alterations in T_{reg} cell function. Our functional assay showed that T_{reg} cells obtained from AD patients with EH retained the suppressive capacity to inhibit proliferation of T_{reg} cells, similar to that of healthy controls. In view of their increased frequency at onset, the net suppressive effects of T_{reg} cells could be maximal during the acute stage of EH. This was reflected in the impairment of γ-interferon production by CD8+ T cells and natural killer (NK) cells in these AD patients with EH. The impaired ability of these cells to produce γ-interferon was restored to levels comparable to those in healthy controls, upon a contraction of T_{reg} cells after clinical resolution. These findings suggest that an increase in T_{reg} cell frequencies would serve to attenuate anti-HSV immune responses necessary for elimination of HSV (fig. 2). This increase in T_{reg} cell frequency would contribute to HSV reactivation by prematurely limiting anti-HSV effector cell responses. Indeed, our immunohistochemical studies demonstrated that increased numbers of Foxp3+ T_{reg} cells occurred in the vicinity of HSV- and VZV-expressing cells in the EH lesions; interestingly, reactivations of VZV can also be seen in some EH lesions (fig. 3). Thus, an increase in the frequencies of T_{reg} cells observed at the onset of EH might be a cause of viral reactivation, but not a mere consequence.

If increased frequencies of T_{reg} cells are responsible at least in part for HSV reactivation in recurrent EH, then a question arises which factors are responsible for driving T_{reg} cell expansions that may induce HSV reactivations. In a recent retrospective analysis of 100 cases of EH, it has been described that 36% of the patients with EH had noted a severe exacerbation of their AD which had typically started 2 weeks before the onset of EH and that the majority of the patients had not received any corticosteroid therapy in the 4 weeks before the onset of EH [7]. A likely interpretation of these

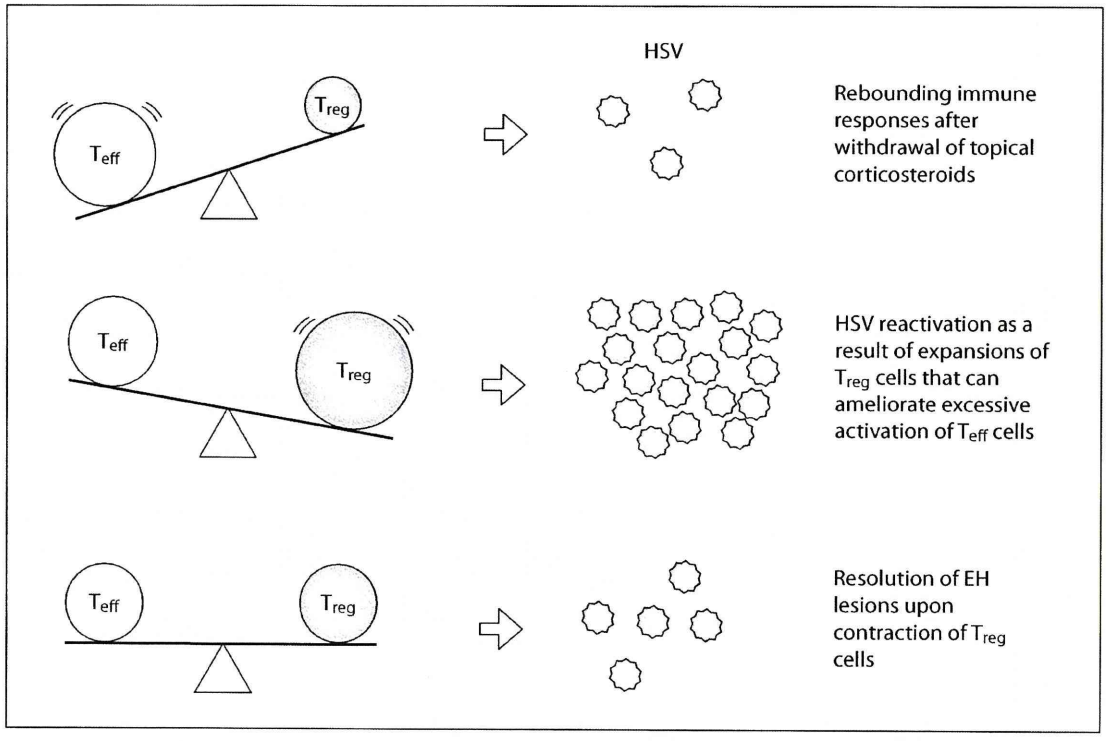

Fig. 2. The balance between T_{reg} cells and antiviral T_{eff} cells before the onset of EH and during the course of EH.

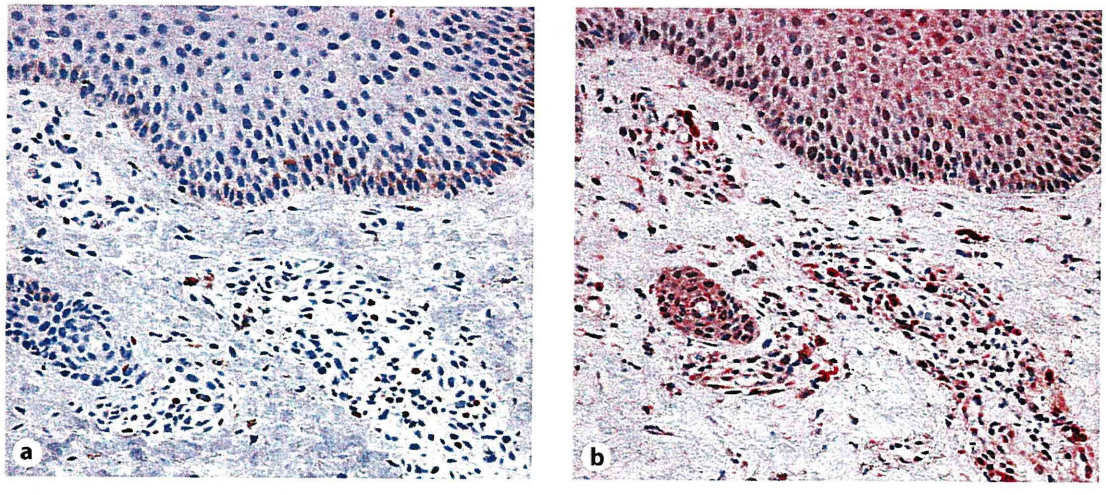

Fig. 3. Positive immunohistochemical staining for T_{reg} cells (**a**) and VZV antigen (**b**) in EH lesions.

observations is that an abrupt shift of host immune responses from a well-controlled state to a robust rebounding, pathogenic inflammatory state would occur upon withdrawal of immunosuppressive agents, such as corticosteroids in AD patients, which could be manifested as an exacerbation of clinical symptoms long before the onset of EH. Probably, this shift would allow latent HSV to be reactivated in an uncontrolled

fashion. According to this scenario, expansions of T_{reg} cells initially required for preventing excessive pathogenic inflammation as a result of withdrawal of topical corticosteroids could in turn contribute to HSV reactivation, resulting in the initiation and progression of EH. These data propose a dual role of T_{reg} cells, either beneficial or harmful, by ameliorating the tissue-damaging effects of antiviral immune responses at the site of inflammation depending on how and when they are expanded. This scenario provides a potential explanation for why apparently disparate data on the role of T_{reg} cells in the pathogenesis of AD have been reported: this is due in part to a neglect in previous studies of evaluating the effects of T_{reg} cells on HSV reactivation.

Decreased Innate Immune Responses in Atopic Dermatitis

Decreased innate immune responses might also explain the susceptibility of AD patients to recurrent viral infections. An aberrant overwhelming Th2-dominated immune response in AD can also be caused by a dysfunction or failure of innate immune responses. In view of the fact that innate immune cells mediate a first line of defense against a wide variety of pathogens and determine the nature of subsequent acquired immune responses to these pathogens [20–22], one can imagine that innate immune cells are functionally defective in patients with AD. Indeed, we previously provided evidence to indicate that NK cells and γδ+ T cells from AD patients are prone to undergo apoptosis upon cell-to-cell contact with activated monocytes and that this preferential apoptosis is the major mechanism responsible for a sustained impairment of Th1 immune responses and increased susceptibility to cutaneous infections, both of which are the hallmarks of AD [23]. It remained unknown, however, whether the functional defect in AD resides fundamentally in the monocytes or the NK cells and γδ+ T cells. In view of recent observations that production of, or responsiveness to, IL-12 is clearly reduced in AD patients [24–26], prime candidates that are defective in AD patients would be a monocyte population rather than NK cells and γδ+ T cells. In support of this possibility, our previous study showed that the removal of monocytes from the culture restored the ability of NK cells and γδ+ T cells from AD patients to produce Th1 cytokines [23].

To determine which arms of innate immune responses are impaired in AD patients, attention should be focused on the fact that AD patients exhibit the increased susceptibility to limited pathogens, *S. aureus*, *Streptococcus pyogenes* and HSV [27–30], while preserving their capacity to mount innate immune responses to other pathogens. These findings suggest that the failure of AD patients to control these infections might result from either absence or inadequacy of innate immune responses to these pathogens, all of which are shown to stimulate Toll-like receptor (TLR) 2 [27, 31–33]. Thus, abnormalities in the TLR2 signaling pathway are likely potential targets in elucidating the pathomechanism of AD. In recent years, attention has been focused on TLR2 pathway defects.

TLR2 can recognize a remarkably broad range of pathogen-associated molecular patterns by homodimerizing as well as heterodimerizing with TLR1 and 6 [34]. Indeed, mice deficient in TLR2 and its downstream effector MyD88 were shown to be highly susceptible to an intravenous inoculation of *S. aureus* [35]. Ahmad-Nejad et al. [36] reported that a missense mutation in the *TLR2* gene R753Q is increased in AD patients with a severer phenotype characterized by markedly elevated IgE antibody levels and increased disease severity which renders these AD patients highly susceptible to *S. aureus* colonization.

We have recently demonstrated that TLR2-mediated proinflammatory cytokine (IL-1β and tumor necrosis factor α) production by monocytes was selectively impaired in AD patients when stimulated with the synthetic TLR2 ligand S-[2,3-bis(palmitoyloxy)-(2-RS)-propyl]-N-palmitoyl-R-Cys-S-Ser-S-Lys_4-OH trihydrochloride [37]: this impairment can be demonstrated with TLR2-mediated stimulation while phorbol-myristate-acetate- or TLR4-mediated proinflammatory cytokine production by the monocytes is not impaired [37]. This selective impairment cannot be explained by differences in cell surface TLR2 expression, because the levels of TLR2 and TLR4 expression on monocytes from AD patients were not significantly different from that in healthy controls [37]. Interestingly, the most remarkable reduction in TLR2-mediated proinflammatory cytokine production by monocytes was observed in the proinflammatory monocytes (CD14dimCD16+HLA-DR+ monocytes) expressing high FcεRI levels from AD patients, but not in those expressing low FcεRI levels [37]. These results suggest that cell surface FcεRI levels on monocytes regulate ongoing innate immune responses to invading pathogens. Therefore, it seems reasonable to speculate that the inhibitory effects FcεRI exerted on TLR2-mediated cytokine production by monocytes could be restored by reducing their FcεRI expression levels. In view of the notion that full activation of TLR2-mediated immune responses might require the assembly of receptor signaling complexes that influence signal transduction [38], the magnitude and consequences of TLR2-mediated immune responses could be influenced by coactivation through other transmembrane receptors. If so, the interplay between FcεRI and TLR2 might be essential in controlling cutaneous microbial infections and host skin inflammatory responses in AD patients. In support of this possibility, we have recently demonstrated that TLR2- and FcεRI-mediated responses counterregulate one another in mast cells [Mizukawa et al., manuscript submitted]. By analogy with our results, monocytes from AD patients heterozygous for the TLR2 R753Q mutation have been reported to produce dramatically less IL-8 than those from patients lacking this mutation [39]. In addition to monocytes, NK cells have been shown to play an important role in regulating ensuing acquired antiviral immune responses: they are shown to negatively regulate the duration and effectiveness of virus-specific CD4+ and CD8+ T cell responses by limiting exposure of T cells to infected antigen-presenting cells [40].

The production of antimicrobial peptides is also an important component of the cutaneous innate immune response. Antimicrobial peptides can directly kill not

only a broad spectrum of microbes including Gram-positive and -negative bacteria, fungi and certain viruses, but also act as a link between innate and acquired immune responses [41, 42]. Human cathelicidin (LL-37) and some defensins have been shown to be chemoattractant for neutrophils, monocytes and T cells [42]. Because LL-37 possesses antiviral activity against HSV and skin from ADEH+ patients exhibits significantly lower levels of LL-37 expression than skin from ADEH– patients [43], a deficiency of LL-37 might render AD patients susceptible to the development of EH.

Conclusion

Viral clearance is a multifactorial process that involves a series of events mediated by T cells, monocytes, dendritic cells, NK cells and B cells, all of which work in combination and in sequence to resolve viral infections. Because each immune cell does not contribute equally to limit viral persistence, it is difficult to explain a special susceptibility to viral infections in atopic subjects solely by a functional defect in a single immune cell population. Possibly the variations in the functional level and/or impact of these immune cells between individuals may explain differences in the ability of each individual to control viral infections. Immune homeostasis in the skin immune system may rely on a delicate balance between the ability of immune cells to react to viral infections and the ability not to react with other harmless microbes, and if so, the imbalance may result in the loss of viral clearance and overwhelming Th2 responses to environmental allergens in AD.

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Relationship between cytomegalovirus reactivation and dermatomyositis

Dermatomyositis (DM) is an autoimmune disease manifested by muscle weakness and characteristic cutaneous eruptions. Cytomegalovirus (CMV) belongs to the β-herpesvirinae subfamily of herpesviridae that cause morbidity and mortality in immunocompromised patients. With respect to the relationship between CMV and DM, it remains unknown whether CMV plays a pathogenetic role or whether CMV disease is an opportunistic infection due to immunosuppressive treatment. We report two patients with DM who developed cutaneous CMV ulcers within one month after the initiation of systemic corticosteroid treatment. In this context, we retrospectively studied the clinical characteristics of six DM patients with CMV reactivation and the effect of corticosteroid treatment on CMV reactivation in these patients. We also examined possible predictive parameters of CMV reactivation during the course of DM. Our results suggest that CMV reactivation occurs more frequently in DM patients than previously recognized; CMV reactivation occurs regardless of the dosage and duration of corticosteroid administration or the presence of underlying disease. Furthermore, our study shows that a reduction in platelets, serum globulin and IgG levels during the course of DM may be useful predictive parameters for CMV reactivation in patients with DM.

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Key words: cytomegalovirus, dermatomyositis, platelet, reactivation

Dermatomyositis (DM) is an autoimmune disease manifested by muscle weakness and characteristic cutaneous eruptions. DM is often associated with malignancy, drugs and infectious agents. Viral infections have been suggested to confer a risk factor for dermatomyositis but so far no convincing data have been reported. Cytomegalovirus (CMV) belongs to the β-herpesvirus family. The initial infection is often asymptomatic and persists in a latent state afterwards [1]. CMV disease is seen in immunocompromised individuals as a primary infection or as a reactivation of latent CMV, including those receiving long-term immunosuppressive agents, organ transplant recipients and patients with acquired immune deficiency syndrome. With respect to DM, it remains unknown whether CMV plays a pathogenetic role [2, 3], whether CMV is related to any clinical symptoms and/or alterations in laboratory findings observed during the course of DM, or whether CMV disease is an opportunistic infection due to immunosuppressive treatment [4-8]. Here we report two patients with DM who developed cutaneous CMV ulcers on the back and anogenital lesions, respectively, within one month after the initiation of systemic corticosteroid treatment. In this context, we retrospectively studied the clinical characteristics of DM patients with CMV reactivation and the effect of corticosteroid treatment on CMV reactivation in patients with DM. We also examined possible predictive parameters of CMV reactivation during the course of DM.

Case 1

An 83-year-old man presented with a 2 month history of a skin rash on his face, chest, back and limbs, associated with myalgia and general fatigue in June 2007. On examination, a violaceous rash was observed on the upper chest and shoulders; Gottron's papules and periungual erythema were also detected on the fingers. Laboratory tests revealed a white blood cell count of $3,400 \times 10^9$/L, hemoglobin 13.2 g/dL, aspartate aminotransferase (AST) 22 IU/L (normal range, 8-33), alanine aminotransferase (ALT) 13 IU/L (normal range, 3-30), creatine kinase (CK) 74 IU/L (normal range, 15-166), and an aldorase (ALD) of 5.4 IU/L (normal range, 2.1-6.1). Anti-nuclear antibodies (ANA) were 40 ×; tests for anti-DNA, -Sm, -RNP and -Jo-1 antibodies were negative. The histology of a biopsy from a violaceous skin lesion showed epidermal atrophy with liquefaction degeneration, and a perivascular lymphocytic and neutrophilic infiltration in the upper dermis which was compatible with the diagnosis of DM. Chest X-ray and a computed tomography (CT) scan of the chest revealed a nodular lesion in the right lung, consistent with a lung malignancy. He was diagnosed as having DM with a lung cancer. This cancer was not treated because of his refusal. He did not want to be treated with systemic corticosteroids for DM on admission, and the laboratory findings, such as CK and ALD, were within normal ranges regardless

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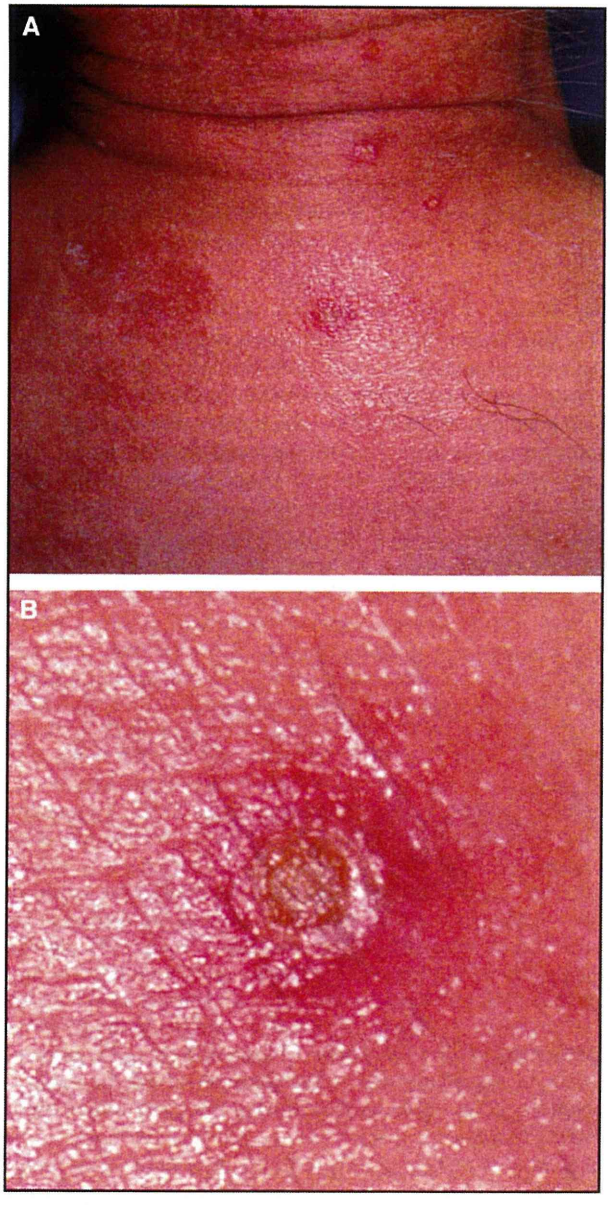

Figure 1. Clinical presentation. **A)** Cutaneous cytomegalovirus ulcers on the back. **B)** Close-up appearance of an ulcer.

of his symptoms; therefore, treatment for DM was not initiated at this stage. Three months later, he presented with severe myalgia, muscle weakness of the limbs, and a deterioration of his skin rash. Abnormal laboratory findings included elevated levels of AST of 59 IU/L, ALT 21 IU/L, CK 417 IU/L, and an ALD of 10.5 IU/L. Because the patient had declined treatment for the cancer, he was given oral prednisolone (PSL) 50 mg daily to treat the DM. Thirteen days later, he developed erythematous ulcerations of 8-10 mm in size with raised borders and excoriations on his neck and upper back *(figures IA, B)*. The cutaneous ulcers were suspicious for CMV disease; examination for CMV was carried out immediately. A scraping obtained from an ulcer on his back revealed the presence of CMV DNA by polymerase chain reaction (PCR); CMV-pp65 antigenemia assay revealed the presence of positive cells. Anti-CMV IgG antibodies were not significantly increased. Treatment with ganciclovir 1800 mg daily i.v. was initiated, resulting in an improvement of the cutaneous ulcers after two weeks of treatment and CMV-pp65 antigens decreased to undetectable levels. PSL was tapered after the control of CMV infection was achieved. Eight months after the initial presentation, the patient died of disseminated intravascular coagulation with metastasis of the lung cancer; at that time, CMV antigenemia was not detected.

Case 2

A 73-year-old man presented with a 5 week history of a violaceous skin rash on the back. Seven weeks earlier, he had noticed muscle weakness of the limbs, myalgia and general fatigue. On examination, Gottron's papules and periungual erythema were absent. Laboratory tests revealed a white blood cell of $4,800 \times 10^9$/L, hemoglobin 14.6g/dL, AST 469 IU/L, ALT 395 IU/L, CK 8659 IU/L, and an ALD of 129 IU/L. Test for ANA was 40×; anti-Jo-1 antibodies were absent. The histology of a biopsy of a violaceous skin lesion showed epidermal atrophy with liquefaction degeneration, and slight perivascular lymphocytic infiltration in the upper dermis which was compatible with the diagnosis of DM. Chest X-ray and CT scan of the chest revealed interstitial pneumonia on the right lower lung. No malignancy was detected by CT scan of the abdomen or gallium scintigraphy. A diagnosis of DM was made and PSL 60 mg daily was initiated. Fifteen days after starting PSL, several 8-10 mm sized ulcers and an erythematous rash appeared in the anogenital area and on the trunk, respectively. CMV antigenemia was detected in the peripheral leukocytes at the same time. Since PSL administration did not significantly alter his clinical symptoms of DM and laboratory parameters, intravenous immunoglobulin G (0.5 g/kg/day for 5 days) and ganciclovir 600 mg daily were started at the same time. The level of CMV antigens as evaluated by CMV-pp65 decreased; however, no significant improvement of clinical symptoms or laboratory parameters was seen. Cyclosporine A 200 mg daily was added to the regimen of PSL 60 mg daily; CMV antigenemia was not detected. PSL and cyclosporine A were gradually tapered after a decrease in serum CK levels was obtained.

Retrospective analysis of clinical and biochemical findings

From the observation of cutaneous ulcers caused by CMV in the above two cases, we performed a retrospective analysis of CMV reactivation in patients with DM, in which CMV reactivation had been investigated independently. Between January 2005 and January 2009, CMV reactivation had been examined in eight DM patients admitted to our hospital (4 males and 4 females, age range 42-83 years, mean age 62.4 years). Diagnosis of DM was made based on the typical

muscle weakness and characteristic cutaneous eruptions. When unexplained clinical symptoms were noticed during the course of DM, evaluations of CMV reactivation were carried out using real-time PCR assays of CMV DNA loads in peripheral leukocytes and/or CMV-pp65 antigenemia assays [8]. Underlying illnesses such as malignancy and interstitial pneumonitis in these patients with DM were examined using X-ray, CT and/or gallium scintigraphy. Treatment with oral PSL was given to all patients; the initial dose was dependent upon the severity of DM.

CMV antigenemia was detected in seven of the eight patients. One patient was excluded from our analysis because of insufficient laboratory data. Therefore, six patients (4 males and 2 females, age range 42-83 years, mean age 64.3 years), including the two cases mentioned above, were enrolled in this retrospective study. The characteristics of the six patients are shown in *table 1*. With respect to the presence of underlying illnesses, malignancy was detected in three of the six patients (Case 1, lung cancer; Case 3, B-cell lymphoma; Case 6, pharyngeal cancer) and interstitial pneumonitis was diagnosed in two patients (Cases 2 and 5). At the time of CMV antigenemia detection, patients had the following: excoriations (3 patients, Cases 1, 3 and 6), erythematous rash (Case 2), skin ulcers (2 patients, Cases 1 and 3), mucous membrane involvement (2 patients, Cases 4 and 5), elevation of hepatic enzymes (2 patients, Cases 1 and 4) and occult blood (2 patients, Cases 3 and 5). All six patients were CMV seropositive; CMV reactivation was not associated with significant levels of CMV-specific antibody response (data not shown). Human immunodeficiency virus testing was negative in all patients. CMV DNA loads in leukocytes were detected in three of the five patients approximately at the same time as the detection of antigenemia. Oral PSL treatment had been initiated in all but one patient (Case 6) at the first detection of CMV antigenemia; no other immunosuppressive agents had been given to those patients prior to the detection of CMV antigenemia. On average, the initial dose of PSL was 0.8-1.2 mg/kg daily. In Case 6, chemotherapy and radiation therapy for pharyngeal cancer had been started without PSL administration at the time of detection of CMV antigenemia. CMV antigenemia was detected less than one month after the initiation of PSL in five out of the six patients. After the detection of CMV reactivation, PSL doses were not altered. The daily dose of PSL was tapered depending on disease severity, as indicated by muscle weakness and the levels of serum CK. All six patients were treated with ganciclovir 600-1,800 mg daily for 3-5 weeks. In Case 5, an improvement of interstitial pneumonitis was reflected by an improvement in the radiological findings.

To determine the effect of PSL treatment on CMV reactivation, 15 patients with collagen diseases or autoimmune bullous diseases who were treated with PSL were enrolled as controls. The initial dose of PSL was 0.8-1.2 mg/kg daily in all control patients: PSL of these patients was tapered gradually to 30-15% of the initial dose over 3 months. No clinical and laboratory findings were detected in any control patient when evaluation of CMV reactivation was carried out. The mean of the total amount of PSL taken by the patients in the control group (9,961.2 mg) was approximately seven times higher than the amount taken by the DM patients with CMV reactivation (1,335.0 mg). Even though the patients in the control group were taking PSL, no CMV antigenemia or CMV DNA loads were detected in the these patients *(table 2)*.

To find predictive factors for CMV reactivation, routinely available laboratory assessments were examined, including hematologic counts (leukocytes, lymphocytes and platelets), total protein, albumin and globulin levels, CK and serum immunoglobulin G (IgG) levels, because investigation of CMV antigenemia and CMV DNA loads are time-consuming. A retrospective analysis of laboratory parameters was examined at four time points: 10-14 days before the detection of CMV antigenemia, at the first detection of CMV antigenemia, when CMV antigenemia became undetectable, and at approximately 2 weeks after the withdrawal of ganciclovir. Serum IgG was evaluated at three time points: 10-14 days before the detection of CMV antigenemia, when CMV antigenemia was detected, and approximately 2 weeks after the withdrawal of ganciclovir. The results showed that the platelet count at the time when CMV antigenemia was first detected was significantly decreased compared to the count prior to detection in all six patients (mean $14.8 \times 10^4/mm^3$ *vs.* mean $24.2 \times 10^4/mm^3$, $p < 0.005$, normal range $15.0\text{-}38.0 \times 10^4/mm^3$) *(figure 2A)*. There was an improvement in the platelet counts 2 weeks after ganciclovir treatment (mean $22.3 \times 10^4/mm^3$). Serum globulin levels were significantly decreased when CMV antigenemia was first detected compared to the levels before the detection of CMV antigenemia (mean 2.87 g/dL *vs.* mean 3.13 g/L, $p < 0.05$, normal range 2.5-3.5 g/dL), and remained lowered except for Case 6 *(figure 2B)*. Serum IgG levels was significantly decreased when CMV antigenemia was first detected compared to the levels prior to detection (mean 995.0 mg/dL *vs.* mean 1,382.5 mg/dL, $p < 0.005$, normal range 778-1,794 mg/dL) in four patients examined, and the levels reduced further in three of the four patients at 2 weeks after ganciclovir treatment (mean 799 mg/dL) *(figure 2C)*. Although the leukocyte, lymphocyte and monocyte counts; and total protein, albumin and CK levels tended to be lower at the time of CMV antigenemia detection compared to the values prior to detection of CMV antigenemia, no significant differences were observed. No atypical lymphocytosis was observed during the detection of CMV antigenemia.

Discussion

Little attention has been paid to the association between DM and CMV reactivation, although there is a report of CMV-induced, progressive interstitial pneumonitis in a DM patient [3]. CMV infection in DM patients undergoing immunosuppressive therapy is considered to be an opportunistic infection [4-8]. In this regard, prolonged administration of a systemic corticosteroid has been recognized as a cause of CMV reactivation because cell-mediated immunity is critical for preventing CMV reactivation. In fact, it has been reported that CMV colitis [9] and retinitis [10] developed after long-term immunosuppressive treatment in patients with DM. However, our study demonstrated that CMV reactivation was frequently observed within one month after the initiation of systemic corticosteroid therapy in DM patients. Consistent with our findings, Fujita *et al.* reported a DM patient who developed

Table 1. Characteristics of DM patients with CMV reactivation.

Patient No.	Age/Gender	Underlying illness	CMV antigenemia	CMV DNA/10^6 leukocytes	Persistence of antigenemia or DNA detection, day	Clinical manifestations at the first detection of CMV antigenemia	Treatment for DM PSL mg daily* (total amount)	Interval** (day)	Treatment for CMV ganciclovir mg daily (day)	Effect of ganciclovir on DM
1	83/M	Lung cancer	+	ND	16	Skin ulcers	50 (650)	13	1,800 (31)	Undefined
		IP				Scratch dermatitis				
						Elevation of transaminase levels				
2	73/M	IP	+	ND	30	Erythematous rash	60 (900)	15	600 (21)	Undefined
						Anogenital skin ulcers				
3	77/F	Lymphoma	+	NT	6	Skin ulcers	60 (1720)	27	1,800 (35)	Undefined
						Scratch dermatitis				
						Occult blood				
4	60/F	None	+	7.6×10^1	14	Oral erosions	35 (2355)	378	900 (21)	Undefined
						Elevation of transaminase levels				
5	51/M	IP	+	1.1×10^3	32	Occult blood	70 (1950)	27	900 (21)	Improvement of radiological findings of IP
6	42/M	Pharyngeal cancer	+	4.1×10^1	28	Oral erosions	NA	10***	1,800 (21)	Undefined
						Scratch dermatitis				

DM: dermatomyositis. CMV: cytomegalovirus. PSL: prednisolone. IP: interstitial pneumonitis. ND: not detected. NT: not tested. NA: not applicable because of no treatment with PSL.
** at the detection of CMV antigenemia*
*** duration from the initiation of PSL to the detection of CMV antigenemia*
**** interval between from the initiation of radiation and chemotherapy to the detection of CMV antigenemia*

Table 2. CMV reactivation in patients who underwent PSL therapy for DM and other illnesses.

	DM	Control
Number of patients	5 (presented in *table 1*)	15
Illness (number of patients)	DM (5)	Lupus erythematosus (2)
		Autoimmune bullous diseases (11)
		Vasculitis (2)
Age (mean, years)	68.8	60.6
(Range)	(51-83)	(18-87)
Gender (M/F)	3/2	6/9
CMV reactivation* (number of patients)	5	0
Total amount of PSL (mean, mg)	1,335.0**	9,961.2**

DM: dermatomyositis. CMV: cytomegalovirus. PSL: prednisolone.
** Detection of antigenemia or CMV DNA.*
*** $P < 0.05$ (unpaired t test).*

CMV gastrointestinal disease after 26 days of PSL treatment [5]. Short-term administration of systemic corticosteroid is unlikely to be the major cause of CMV reactivation. Moreover, no CMV antigenemia or CMV DNA load was detected in patients in the control group which had a significantly higher mean total dose of PSL compared the mean total dose in DM patients. Because systemic corticosteroids were given only for 4 weeks in these patients, it is implausible that the CMV reactivation would be caused by such a short exposure to systemic

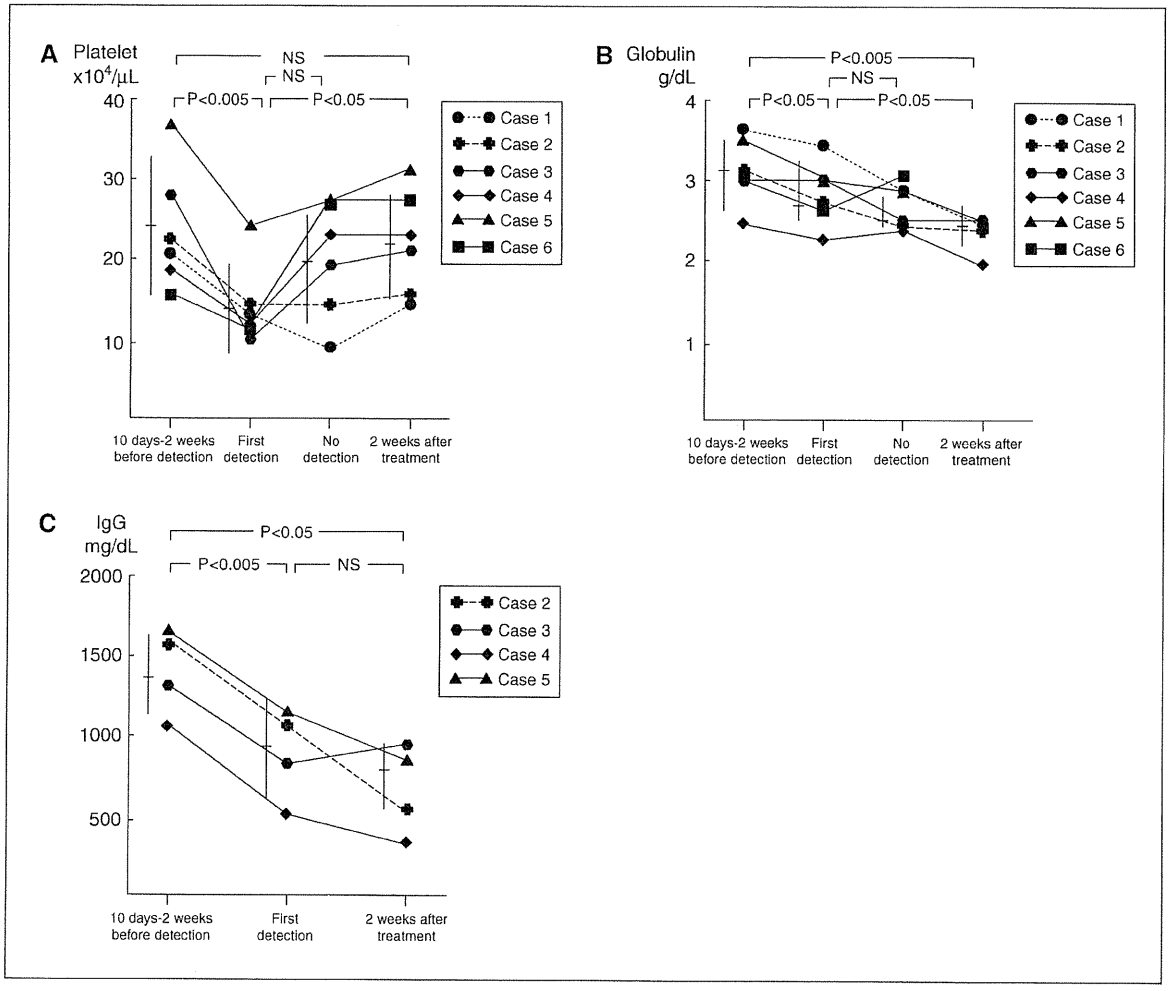

Figure 2. Alterations in parameters. **A)** Platelet count. **B)** Serum globulin level. **C)** Serum immunoglobulin G level.

corticosteroids. Under such a short exposure to systemic corticosteroids, DM patients are more susceptible to CMV reactivation compared to other patients.

It remains unknown whether CMV contributes to the pathogenesis of DM or not. It has been shown that the CMV genome encodes a series of genes that are homogenous to cellular genes, and the host response to viral determinants may cross react with host tissues, eventually leading to autoimmunity [11]. Recently, Fasth *et al.* suggested that CMV might play a role in propagating DM in a subset of patients, by showing that $CD28^{null}$ T cells dominated in their muscles; circulating $CD28^{null}$ T cells were significantly more frequent in CMV seropositive individuals, they responded to CMV antigen stimulation, and correlated with disease duration [12]. Indeed, in our study, when unexplained symptoms and/or alterations in laboratory findings, such as skin ulcers, excoriations and elevation of hepatic enzymes, were observed during the course of DM, CMV reactivation was detected. These observations seem to provide an explanation for the atypical symptoms and/or alterations in laboratory findings observed during the course of DM. The improvement in the radiological findings of interstitial pneumonitis in Case 5 was obtained after the anti-CMV treatment with ganciclovir. These findings also support that the increase in disease activity of DM might be related to the CMV reactivation.

Our study revealed significant decreases in platelet counts, serum globulin and IgG levels at the time of detection of CMV antigenemia in our series of DM patients. Although thrombocytopenia caused by CMV infections is well known [13], alterations in platelet counts, serum globulin and IgG levels in DM patients in the setting of CMV reactivation have not been reported. After ganciclovir was administered, the platelet count improved significantly. Therefore, it is possible that the platelet count reduction is strongly linked to CMV reactivation. Significant decreases in serum globulin and IgG levels may be interpreted as the effect of systemic corticosteroid administration. Although there is a prominent decrease in leukocyte counts during CMV antigenemia in patients with drug-induced hypersensitivity syndrome [14], a decrease in the leukocyte count was not detected in this study. Thus, a decrease in the platelet count, serum globulin and IgG levels observed at the time of detection of CMV antigenemia may be useful predictive parameters for CMV reactivation during the course of DM patients, although the exact mechanism by which CMV causes these alterations remains unknown. In view of the fact that CMV disease may be fatal [5, 14], and that the measurement of CMV antigenemia and CMV DNA in leukocytes is time-consuming, monitoring of these parameters may lead to the early recognition of CMV disease during the course of DM.

In conclusion, our results demonstrated that CMV reactivation might be more frequent in patients with DM than previously recognized, and unexplained clinical symptoms, such as scratch dermatitis and skin ulcers, observed under a short exposure to systemic corticosteroids, might be relevant to CMV reactivation in patients with DM. Our findings suggest that alterations in laboratory findings during the course of DM may be associated with CMV reactivation. Therefore, the assessment of the platelet count, serum globulin and IgG levels during the course of DM may serve as indirect parameters for CMV reactivation. ■

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12

Drug-induced Hypersensitivity Syndrome

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Introduction

The first description of drug-induced hypersensitivity syndrome is generally credited to Meritt and Putmam, who in 1938 reviewed the toxic symptoms caused by phenytoin and noted that the symptoms could be divided into two cutaneous reactions: the first one being a mild, mobilliform eruption that healed upon withdrawal of phenytoin and often did not recur, and another type being a severe exfoliative dermatitis with fever and eosinophilia. Since then it has become clear that the second reaction is also associated with lymphoadenopathy and multivisceral involvement such as hepatitis; and Chaikan et al. were the first to describe the systemic implication of this reaction. In 1988, Shear and Spielberg coined the term 'hypersensitivity syndrome' to refer these diverse entities. The term 'hypersensitivity syndrome' could encompass many different entities, including drug allergy and a genetic defect of drug metabolism. Bocquet et al. introduced the term 'drug reaction with eosinophilia and systemic symptoms (DRESS)' for this syndrome to distinguish it from other severe drug reactions which are not associated with eosinophilia. This syndrome was recognized as a distinct disorder in the early 1960s. Although the term is still used occasionally to describe the clinical symptoms in Europe, it has fallen into disuse, because in 1998 we proposed the alternative term 'drug-induced hypersensitivity syndrome' based on a case study of patients who developed a more severe forms of this syndrome associated with reactivation of human herpesvirus 6 (HHV-6)[1,2]. Because a retrospective nationwide survey of patients in Japan revealed that reactivations of HHV-6 were detected in the vast majority of patients who satisfy not only our tentative criteria for DIHS but also those reported by Bocquet et al., we, a Japanese consensus group, established a set of criteria for diagnosis of DIHS in 2006, including HHV-6 reactivation. Although the clinical and laboratory features of this syndrome in its florid form are currently well recognized in Japan, there has been much debate about the inconsistent and variable terminology. While HHV-6 reactivation can be widely used as a specific and sensitive diagnostic clue in Japan, the validity has not necessarily been confirmed in other parts of the world largely due to an extraordinarily low prevalence of this test. Thus, it remains unknown whether DIHS and DRESS could be part of the continuum of the same disease; that is, DRESS could represent a condition including a clinically mild form of this syndrome.

In this review, we consider the epidemiology, diagnosis, pathogenesis and treatment of this syndrome.

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Epidemiology

DIHS has a worldwide distribution but it is undoubtedly underdiagnosed in many countries due to unawareness of this syndrome. This syndrome is much more common

than Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), whose incidence are estimated at 1 to 6 cases and 0.4 to 1.2 cases per million-years, respectively[3]; its true incidence, however, remains unknown because its variable presentation and diverse clinical features and laboratory abnormalities may have resulted in inaccurate diagnosis and reporting. Nevertheless, since clinical manifestations and laboratory finding of this syndrome in its florid form has become widely recognized in Japan, the reported incidence has risen sharply, during the time between 2006 and 2008 and the incidence could be estimated at >10 cases per million years. Many reports generally agree that DIHS has no age or sex prediction while, in SJS/TEN, women are about 1.5 times more likely to be effected than are men. In Japan, the incidence increases steadily with advancing age. Our recent unpublished study has shown that median age at diagnosis was about 51.4 years for men and 55.7 years for women (range 0-83); only 7% of patients were younger than 20 years. It remains unknown whether a racial predilection could exist; although it has been reported that occurrence of the disease is twice as high in elderly black men, but the cause of this skewed distribution is unknown. There were no seasonal variations. Our series with this syndrome showed no increased incidence of a personal or family history of atopy and drug eruption. Most cases are sporadic. About half of the patients have had a infection within the previous 1 month, most commonly viral infections, although the responsible pathogen is not identified.

Diagnosis

The diagnosis of this syndrome is not difficult for Japanese dermatologists who are familiar with the diagnostic criteria we established in 2006 (Table 1). However, the diagnosis may be challenging for the doctors who are not familiar with the criteria and may not have experienced cases. Diagnostic criteria for DIHS established by us, named the Japanese Research Committee on Severe Cutaneous Adverse Reaction (J-SCAR) differ so much from those for DRESS established by European group in that

Table 1. Diagnostic Criteria for DIHS Established by a Japanese Consensus Group[7]

1. Maculopapular rash developing >3 weeks after starting with a limited number of drugs
2. Prolonged clinical symptoms after discontinuation of the causative drug
3. Fever (>38°C)
4. Liver abnormalities (ALT>100 U/L)*
5. Leukocyte abnormalities (at least one present)
a. Leukocytosis (>11x10^9/L)
b. Atypical lymphocytosis (>5%)
c. Eosinophilia (>1.5x10^9/L)
6. Lymphademopathy
7. HHV-6 reactivation

The diagnosis is confirmed by the presence of the seven criteria above (typical DIHS) or of five of the seven (atypical DIHS).
*This can be replaced by other organ involvement, such as renal involvement.

HHV-6 reactivation is included in the former criteria, but not in the latter.

Diagnosis of definite or typical DIHS requires the presence of the seven criteria. Probable or atypical DIHS can be diagnosed in patients with typical presentation (criteria 1-5) but in whom HHV-6 reactivation cannot be detected, probably because of inappropriate timing of sampling. Importantly, our series of >60 patients diagnosed by clinical findings has consistently shown that HHV-6 reactivation can be detected in the vast majority of patients who satisfy the other six criteria (criteria 1-6) and show clinical manifestations consistent with those reported by Bocquet et al., but not in those with other types of drug eruption such as SJS/TEN. In contrast, HHV-6 reactivation is rarely detected in patients with a tendency toward milder forms of this syndrome. This finding was confirmed by Tohyama et al.[4].

Exclusions now include measles, infectious mononucleosis, Kawasaki syndrome, hypereosinophilic syndrome, drug-induced pseudolymphoma, and staphylococcal toxic shock syndrome[5]. Specific features of this syndrome can often be misdiagnosed as bacterial infection which may place a substantial burden on physicians to consider empirical antibiotic therapy in patients with unexplained illness, such as rash and fever, following wide acceptance

of the criteria, there has been little disagreement among Japanese dermatologists about the diagnosis of this syndrome with obvious findings. However, we should bear in mind that the clinical criteria are not all present on any given day, particular at onset, and that the severity of these clinical symptoms at onset provide only a guide to prognosis and is not absolute: usually patients initially develop two or three feature of this syndrome followed by a step-wise development of other symptoms. Because eosinophilia is seen at most in 60-70% of patients who satisfy the criteria, we proposed that the tern DRESS be replaced by the term DIHS to avoid confusion due to the lack of consensus in the literature about its terminology. HHV-6 reactivation as evidenced by the significant rise in HHV-6 IgG titers or HHV-6 DNA loads should be used to confirm a clinical diagnosis rather than simply as a screening tool, because this reactivation can be detected at the certain timing, 2-3 weeks after onset or withdrawal of the causative drug, despite such a wide variety of clinical symptoms.

Clinical Features

This syndrome typically occurs with fever or cutaneous lesions 3 weeks to 3 months after starting therapy with a limited number of drugs, mainly anticonvulsant drugs (Table 2). It remains unknown, however, why a limited number of drugs can cause this syndrome because they do not have common epitopes and common pharmacologic actions. Depending on race, significant differences exist among these drugs with regard to the potential to cause DIHS: for example, in Japan minocycline rarely causes DIHS while mexiletine frequently does, and this is precisely the opposite of that happened in Caucasians. Cross-reactivity among these drugs has been frequently reported, because phenytoin, phenobarbital and carbamazepine can be metabolized to hydroxylated aromatic compound and arene oxides are suggested intermediates in the reaction[6]. 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: indeed, we have never seen patients who developed DIHS within 2 weeks after starting therapy. In contrast, DIHS has ever been reported to develop in patients receiving anticonvulsants for up to 40 years. There might be a prodrome suggestive of upper airway infections.

Table 2. Drugs Frequently Causing DIHS/DRESS

• Carbamazepine	• Dapsone
• Phenytoin	• Salazosulfapyridine
• Phenobarbital	• Allopurinol
• Mexiletine	• Minocycline

The maculopapular or erythematous eruptions are initially observed or the face, upper trunk and upper extremities: they may be slightly pruritic and can become confluent. One of the characteristic features of the eruption at the early stage is facial, periorbital, or neck erythema and edema studded with pinhead-sized pustules, reminiscent of acute generalized exanthematous pustulosis. Although some erythematous macules may coalesce to form blisters, most of erythematous macules do not evolve into blisters and no mucous membrane involvement is usually seen, findings that can be used to differentiate this syndrome from SJS/TEN. Follicular accentuation of the erythematous papules is often observed in the early stage of the rash. The eruption often generalizes into exfoliative dermatitis or erythroderma, which usually occur with continued use of the causative drug after onset of the rash. Fever usually precedes the rash by several to a few days and temperature ranges from 38 to 40°C with spikes that may generate concern regarding an underlying infection. Tender lymphadenopathy can be seen in most (>70%) patients, particularly early in the illness, affecting predominantly cervical nodes. Bilateral swelling of the salivary glands with severe to mild xerostomia has been seen early in the illness. Importantly, more severe reactions (Fig. 1) often occur 3-4 days after withdrawal of the causative drug: this paradoxal worsening of clinical symptoms after withdrawal of the causative drug is also characteristic of DIHS and may be mistaken as severe infectious diseases by physicians of first contact to such a case, causing

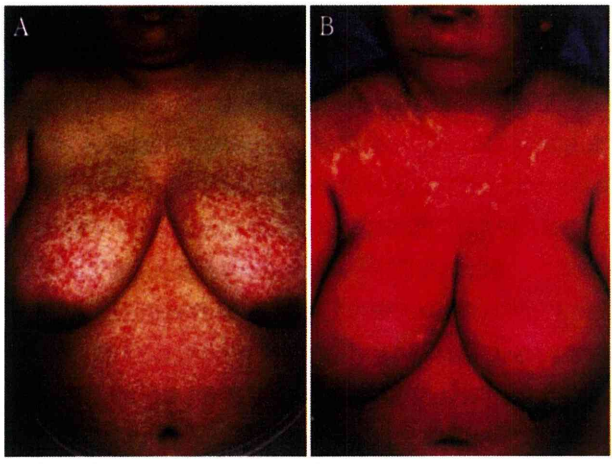

Fig. 1. The patient's chest and abdomen on her initial presentation and 4 days after withdrawal of the drug. (A) On her initial presentation, slight erythema can be seen. (B) A dramatic deterioration of her symptoms is observed despite withdrawal of the drug.

high levels of suspicion of infection: upon the suspicion, unnecessary empirical antibiotic therapy may be started, often resulting in the development of additional drug hypersensitivity. This is because patients with DIHS often show unexplained cross-reactivity to multiple drugs with different structures, including those used after onset of symptoms. Such clinical variability in the presentation allows for a delay in diagnosis, which can lead to significant morbidity.

Liver abnormalities occur usually in the early phase in up to 70% of patients and are characterized by a marked increase in serum alanine aminotransferase (ALT). Hepatomegaly accompanied by splenomegaly is frequently observed. In other patients, various forms of renal involvement have also been reported. Depending on the drug, involvement of other organs varies: hepatitis is often observed in phenytoin-, minocycline-, or dapson-induced DIHS[5], while renal involvement is particularly evident in allopurinol-induced DIHS[5]. In many severe cases, these variable symptoms continue to deteriorate or several flare-ups can be seen even for weeks after stopping the causative drug. Although pulmonary involvement is rarely reported in patients with DIHS, interstitial pneumonia with eosinophilia is often observed in patients receiving minocycline[5]. Myocarditis can also develop at onset of DIHS or approximately 40 days after onset: clinical symptoms suggestive of myocarditis include heart failure symptoms such as chest pain, unexplained tachycardia, breathlessness and low blood pressure during the early phase of disease course. Other features of DIHS include coronary artery thrombosis, and encephalitis[7]. We have recently reported a patient with DIHS who developed limbic encephalitis and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) long after resolution of rashes[8]: HHV-6 has been suggested to be involved in the limbic encephalitis associated with hyponatremia, although HHV-6 DNA was not detected in the cerebrospinal fluid. We have also seen patients with DIHS who developed viral meningitis and herpes zoster 1-2 months after resolution of rashes. Thus, depending on the sites and severity of organ damages, various clinical symptoms would develop at various time points after onset. Nevertheless, their development may be clinically silent and recognized only months and years later in some patients.

Laboratory Findings

This syndrome is characterized by leukocytosis with atypical lymphocytes and eosinophilia of various degrees. Leukopenia or lymphopenia has been also reported[9] and this occasionally precedes leukocytosis. The lymphocytosis is primarily due to an increase in either CD4 or CD8 T-cell counts. Eosinophilia may often be delayed for 1 to 2 weeks and occur even after elevations in liver enzyme return to baseline. Elevations in liver enzymes usually persist for several days or weeks after withdrawal the causative drug but in the vast majority of patients the hepatitis resolves spontaneously, although hepatic necrosis may cause death in the setting of coagulopathy and sepsis[5]. The mortality from DIHS can be approximately 10% in our case series and has been correlated with the degree of renal involvement rather than hepatic involvement[7].

A dramatic decrease in serum IgG, IgA, and IgM levels is typically observed at onset and the lowest levels are usually detected a week after withdrawal of the causative drug. One to 2 weeks after the nadir in the decrease, the overshoot in Ig levels is transiently observed and their levels eventually return to normal upon full recovery (Fig.