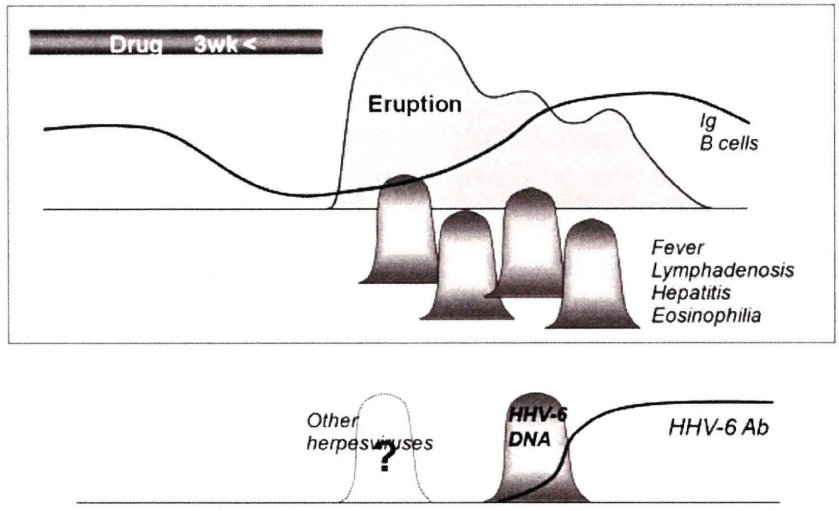

Fig. 2. The clinical course of DIHS[7]. This syndrome usually begins with a fever shortly followed by a maculopapular rash >3 weeks after starting therapy with a limited number of drugs, such as anticonvulsants. Patients usually develop two or three features of symptoms followed by a step-wise development of other symptoms. These symptoms continue to deteriorate or several flare-ups can be seen even for weeks or months after stopping the offending drug. Serum Ig levels continue to decrease for a weak after withdrawal of the drug. Despite such a wide variety of clinical symptoms, HHV-6 reactivation occurs 2-3 weeks after onset.

2). Nevertheless, our follow-up analyses of serum Ig levels long after resolution showed that their serum Ig levels tend to be lower than those in healthy controls.

Although HHV-6 was previously thought to be the only herpesvirus reactivated during the course of DIHS[1], our recent studies of real-time measurement for viral loads have demonstrated that other herpesviruses, such as Epstein Barr virus (EBV), HHV-7, and cytomegalovirus (CMV), are also reactivated during the course of the disease in a sequential order as demonstrated in graft-versus-host disease (GVHD)[10]. Nevertheless, because HHV-6 reactivation can be detected in the vast majority (>70%) of patients with DIHS at a certain time point, 2-3 weeks after onset but not in those with other drug eruptions, this becomes a gold standard test for identifying patients with DIHS in Japan[11]. According to our sequential analyses of viral loads in patients with DIHS, the cascade of reactivation events initiated by HHV-6 or EBV extends, with some delay, to HHV-7 as well, and eventually to CMV[10]. In view of the similarity between DIHS and GVHD with regard to the clinical manifestations and the order of viral reactivations, frequent deterioration or several flare-up of clinical symptoms occurring after withdrawal of the causative drug in DIHS could be explained by sequential reactivations of herpesviruses in other organs which would also occur in a sequential order but totally independent of that occurring in the blood.

Pathological Findings

The histologic picture of DIHS is superficial perivascular lymphocytic infiltrates composed mainly of T cells with some extravasated erythrocytes or eosinophils. Depending on biopsied lesions, variable degrees of focal spongiosis associated with a lichenoid infiltrate can be seen, but severe epidermal damage as frequently seen in SJS/TEN is never detectable. In some patients with DIHS, a noncaseating epithelioid granulomatous infillrate or a pseudolymphoma-like infiltrate can be detected in the upper or mid dermis.

In Vitro Testing

Lymphocyte transformation test (LTT) is a reliable method to define the causative drug in drug eruptions, when performed at the right timing[12]. In patients with

DIHS, false negative LTT reactions were constantly observed when examined at the acute stage, usually within 2-3 weeks after onset, regardless of whether the patients are on therapy with systemic corticosteroids. Positive LTT reactions were observed when tests were performed after resolution, usually 5-8 weeks after onset[12]. In contrast, totally opposite results were observed in patients with SJS/TEN: positive LTT reactions were observed when tests were performed in the acute stage but not after resolution.

Genetics

There have been attempts to link susceptibility to DIHS to allelic variations. However, although a strong association has been demonstrated between human leukocyte antigen (HLA)-B*1502 allele and carbamazepine-induced SJS/TEN in Ham Chinese patients[13], only small cohorts of patients have been analyzed in association studies in which the frequencies of the alleles of interest have been compared between DIHS and control individuals[14,15]. Although the insufficient sample sizes in this study preclude any general conclusions, our results showed that the HLA-B*1502 allele was not observed in any patients with DIHS or SJS/TEN caused by anticonvulsants and that the HLA-B*4801 allele was found in 6 out of the 16 patients with DIHS (37.5%): the frequencies of the HLA-B*4801 allele in patients with DIHS was considerably higher than the reported frequencies in the Japanese population (4.3%), although the difference is not statistically significant after correction for multiple comparisons[15]. Four out of the 6 patients with HLA-B*4801 allele showed severe liver dysfunction (ALT >300 IU/l) during the course of DIHS. In addition, 4 out of the 16 patients with DIHS had the HLA-B*1301 allele (25%); the frequency of the HLA-B*1301 allele in those patients were much higher than that reported for Japanese population (1.3%), although not statistically significant. Interestingly, in 3 out of the four patients with HLA-B*1301, not only HHV-6 but also CMV was reactivated in association with severe liver dysfunction during the course of DIHS. In view of this possible association between the HLA-B*1301 allele and particular virus reactivation in our study, the effect of certain HLA-B alleles on the occurrence of virus reactivations may contribute, at least in part, to the HLA-B allele association with the disease. Large-scale studies will be necessary for full investigation of candidate alleles.

Pathogenesis

Activated T cells seem to play an important role in DIHS, as suggested in other severe drug eruptions. It was previously believed that DIHS merely represents an exaggerated, hyperinflammatory response with inflammation-induced viral reactivations and subsequent organ injury. It has now become clear, however, that immune homeostasis in the skin relies on a delicate balance of effector T (Teff) cells and regulatory T (Treg) cells and this balance is disturbed in DIHS. Our recent study has clearly demonstrated that dramatic expansions of functional Treg cells were found in the acute stage of DIHS; in contrast, such expansions were never observed in the acute stage of SJS/TEN but their capacity to suppress the activation of Teff was profoundly impaired[16]. This expansion of Treg cells would occur in an unrecognized fashion to counteract Teff-induced inflammation far before onset of DIHS, which would contribute to not only the delayed onset, but also to viral reactivations (Fig. 3). Eventually, however, the delicate balance would be disturbed in favor of Teff cells despite protracted expansions of Treg cells, resulting in an inflammatory outcome at onset of DIHS. Thus, immune responses during the acute stage of DIHS are characterized by a complex interplay among Teff cells, Treg cells and herpesviruses: Teff populations consist of anti-viral Teff and anti-drug Teff cells that are dominated by a Th2 phenotype due to their relative resistance to the action of Treg cells. The expanded Treg cells would also limit the severity of Teff-mediated immunopathology. This scenario provides an explanation for why severe epidermal damage cannot be detected in the skin lesions of DIHS, unlike SJS/TEN lesions, why the onset of DIHS is delayed in relation to the introduction of the causative drug, and why proliferation of drug-specific Teff cells as evidenced by a positive LTT

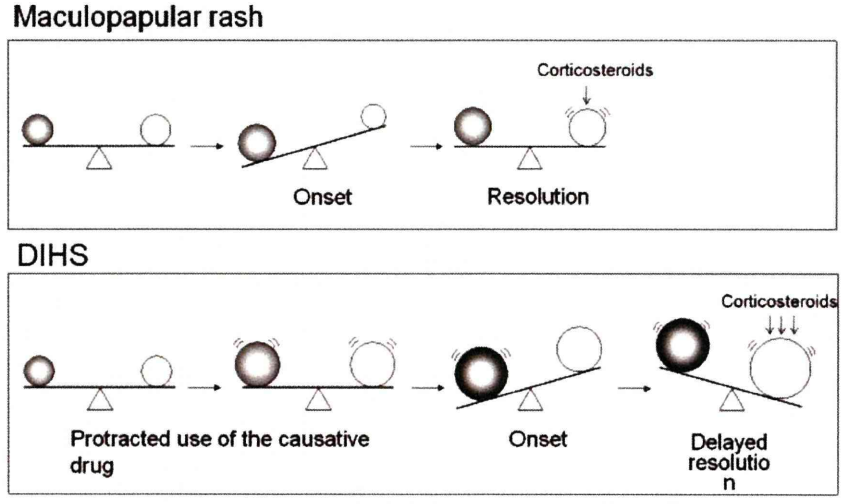

Fig. 3. A hypothetical model for the development of DIHS. In quiescent conditions, the action of Teff cells is under control of Treg cells. Protracted use of anticonvulsants with potentially immunosuppressive activities results in expansions of Treg cells, which serve to inhibit activation of Teff cells. Eventually, however, a delicate balance between Teff and Treg cells is disturbed, leading to onset of DIHS. Systemic corticosteroids can improve clinical symptoms, probably by their potentiating Treg cell function.

reaction can not be detected at the acute stage.

Surprisingly, Treg cells expanded at the acute stage of DIHS gradually lose their original suppressive activity associated with their contraction after full recovery, although they are present in normal frequency: such a gradual loss of Treg cell function after the resolution of DIHS may result from their eventual exhaustion by persistent or repeated viral reactivations. Indeed, several autoimmune diseases have been reported to occur at intervals of several months and years after clinical resolution of DIHS: they include type 1 diabetes mellitus, thyroiditis, SLE and sclerodermoid GVHD-like disease[17,18].

Treatments

Early recognition of this syndrome is the most important step in treatment and is essential in improving patient outcomes, because many physicians are not familiar with this syndrome. Empirical treatment with antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) should not be done even in DIHS patients with a high-grade fever during the acute stage, which may confuse or worsen the clinical picture probably due to unexplained cross-reactivity to multiple drugs a finding frequently seen in patients with DIHS[19].

Despite the lack of large controlled trials, corticosteroids are the immunosuppressive agent most frequently used for the treatment of DIHS and the most consistently effective: the usual dosage is prednisolone 40-60 mg/day. They are administered for 2-3 months: systemic corticosteroids need to be tapered over 6-8 weeks to prevent the relapse of various symptoms of this syndrome. In this regard, we have recently reported two patients with DIHS who subsequently developed cutaneous and gastrointestinal CMV ulcers: in one patient fatal CMV enterocolitis developed soon after tapering the done of oral prednisolone[20]. Because our retrospective studies showed that older and male patients with antecedent high HHV-6 DNA loads are at risk of subsequently developing CMV disease that may be fatal, patients with DIHS treated with corticosteroids should be monitored carefully for the development of CMV disease.

Conclusions

Despite substantial progress during the past decade in uncovering the cellular and molecular mechanisms mediating cutaneous immune homeostasis in DIHS,

translation of basic research findings into more effective monitoring and management of patients with DIHS has been relatively slow. Another important outcome measure is the risk of subsequent induction of autoimmune disease as sequlae of DIHS. Future progress in disease monitoring and intervention will depend on how we can develop a more integrated understanding of the mechanisms.

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Possible involvement of CD14+ CD16+ monocyte lineage cells in the epidermal damage of Stevens–Johnson syndrome and toxic epidermal necrolysis

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Conflicts of interest
None declared.

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Summary

Background Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are characterized by keratinocyte apoptosis and necrosis, resulting in epidermal detachment. Although monocytes abundantly infiltrate the epidermis in SJS/TEN skin lesions, the properties and functions of these cells have not been fully examined.

Objectives To determine the properties of monocytes infiltrating into the epidermis in SJS/TEN.

Methods Immunostaining of skin sections was performed to examine the membrane markers of monocytes infiltrating into skin lesions.

Results Immunostaining of cryosections from 11 SJS/TEN skin lesions revealed numerous CD14+ monocytes located along the dermoepidermal junction and throughout the epidermis. The cells coexpressed CD16, CD11c and HLA-DR. CD14+ CD16+ cells were identified in very early lesions without epidermal damage, suggesting that their infiltration is a cause, rather than a result, of epidermal damage. Moreover, these cells expressed CD80, CD86 and CD137 ligand, indicative of their ability to facilitate the proliferation and cytotoxicity of CD8+ T cells. CD16+ cells infiltrating the epidermis and detected at the dermoepidermal junction were immunostained and counted in paraffin-embedded skin sections obtained from 47 patients with drug rash manifested as TEN, SJS, maculopapular-type rash or erythema multiform-type rash. The number of CD16+ monocytes infiltrating the epidermis increased significantly, depending on the grade of epidermal damage.

Conclusions These findings suggest that the appearance of CD14+ CD16+ cells of monocyte lineage plays an important role in the epidermal damage associated with SJS/TEN, most probably by enhancing the cytotoxicity of CD8+ T cells.

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are both characterized by epidermal cell death and separation of the skin at the dermoepidermal junction, which confers the characteristic appearance of scalding.[1] In SJS/TEN, keratinocyte cell death is the result of apoptosis and necrosis.[2] Studies further examining the mechanism of epidermal cell death in these related diseases suggest a role for soluble factors in their pathology. For example, high levels of soluble tumour necrosis factor (TNF)-α, Fas ligand and granulysin were detected in the blister fluid of patients with TEN.[3–5] In particular, granulysin is a key mediator that causes rapid and disseminated skin cell death.[5] This fluid also contains CD8+ T cells,[6–8] which express high levels of perforin and granzyme B.[8] Support for an important role for these molecules comes from a study showing that the cytotoxic effects of CD8+ T cells against keratinocytes can be enhanced by treating keratinocytes with interferon (IFN)-γ, and can be reduced by blocking the perforin/granzyme B pathway.[8]

In this study, we examined the monocytes in SJS/TEN. These cells abundantly infiltrate the epidermis and accumulate at the dermoepidermal junction in SJS/TEN skin lesions.[9–11] Earlier studies indicated that monocytes play an important role in the pathogenesis of SJS/TEN. Roujeau *et al.*[9] analysed cell suspensions derived from the epidermis of patients with TEN and found that approximately 80% of the blood cells contained in the cell suspensions expressed monocyte markers.

Using immunohistochemical analysis, Paquet *et al.*[10] showed that monocytes were the predominant infiltrating cell type in epidermal lesions and that within the epidermis they were present in higher numbers than T cells. The same authors showed that these monocytes expressed high levels of TNF-α, suggesting that they are involved in keratinocyte apoptosis.[11] However, the roles of monocytes infiltrating SJS/TEN lesions remain poorly understood.

Monocytes consist of two populations: a large population of CD14+ CD16− monocytes and a minor population of CD14+ CD16+ monocytes.[12] Here, we demonstrate that monocytes of the CD14+ CD16+ lineage abundantly infiltrate both the epidermis and the dermoepidermal junction of SJS/TEN skin lesions. Additionally, we show that these cells express CD11c, HLA-DR, costimulatory factors such as CD80 and CD86, and CD137 ligand (CD137L), which has an important role in the proliferation and cytotoxicity of CD8+ T cells.[13,14]

Materials and methods

Skin samples

Skin biopsies for cryosectioning and diagnostic studies were taken from four patients with TEN and seven with SJS (Table 1). On admission, informed consent was provided by all patients. At the time of the biopsy, patient 3 had been treated with systemic corticosteroid for 7 days; however, the area of epidermal detachment had been expanding. None of the other patients had received systemic corticosteroids prior to the biopsy. Additionally, paraffin-embedded tissue blocks were prepared from skin biopsies obtained from 47 patients with drug rash, including 13 with TEN, nine with SJS, and 25 with maculopapular-type (MP) or erythema multiform-type (EM) drug rash. These samples were obtained within 5 days after the onset of MP, EM or epidermal detachment, and prior to systemic therapy. Additionally, normal-appearing skin adjacent to the lesional skin was biopsied in eight patients with SJS/TEN.

Immunofluorescence and immunohistochemical staining

For immunofluorescence double staining, cryosections (5 μm) embedded in optimal cutting temperature compound (Sakura Finetechnical Co., Tokyo, Japan) were fixed in cold acetone for 3 min, washed, and then incubated with rat anti-CD16 antibody (GeneTex, Irvine, CA, U.S.A.). Donkey antirat antibody labelled with Alexa Fluor 594 (Invitrogen Japan, Tokyo, Japan) was used as secondary antibody. After a second washing step, the sections were incubated with antibodies against CD2 (Dako Japan, Tokyo, Japan), CD11c (BD PharMingen, Tokyo, Japan), CD14 (Zymed Laboratories, South San Francisco, CA, U.S.A.), CD163 (Abcam, Cambridge, U.K.), CD56 (Dako Japan), CD80 (Abcam), CD86 (BioLegend, San Diego, CA, U.S.A.), CD137L (Abcam) or HLA-DR (Dako Japan) and with antimouse or antirabbit antibody labelled with Alexa Fluor 488. The same protocol was followed for CD8 (Abcam) and CD137 (Abcam), with costaining again using antimouse or antirabbit antibody labelled with Alexa Fluor 488. Fluorescence was observed under a fluorescence microscope (Nikon, Tokyo, Japan).

For immunohistochemical staining, the cryosections were processed as follows: endogenous peroxidase was quenched, nonspecific binding sites were blocked, and the sections then treated with monoclonal antibodies against HLA-DR (Dako Japan) and CD16 (Dako Japan). Immunostaining was then carried out using Histofine Simple Stain MAX PO (Nichirei

Table 1 Characteristics of patients with Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN)

Patient/age (years)/sex	Diagnosis	Causative drug	Duration of disease prior to biopsy (days)	Area of epidermal detachment (%)		Area of skin rash (%)	Mucosal involvement	Treatment/ prognosis
				At the first visit	Max			
1/74/F	TEN	Antibiotic/NSAID	5	60	100	100	E, M, G	mPSL, glb/D
2/82/M	TEN	Antibiotic	2	40	95	95	M, G	mPSL, glb/A
3/78/F	TEN	Allopurinol	7	35	40	40	E, M, G	mPSL, glb/A
4/91/F	TEN	Allopurinol	9	30	30	70	E, M, G	mPSL/A
5/40/M	SJS	Anticonvulsant/ NSAID	1	1	5	70	E, M, G	mPSL, glb/A
6/70/F	SJS	Mexiletine	1	1	5	40	M, G	mPSL/D
7/56/F	SJS	Antibiotic	2	1	1	30	M, G	mPSL/A
8/72/M	SJS	Allopurinol	2	1	1	15	E, M, G	mPSL/A
9/26/F	SJS	Anticonvulsant	2	1	1	5	M, G	PSL 30 mg/A
10/82/F	SJS	NSAID	5	1	1	1	E, M, G	PSL 40 mg/A
11/84/F	SJS	Allopurinol	6	1	1	1	M, G	mPSL/A

A, alive; D, death from sepsis and multiple organ failure; E, eyes; G, genital; glb, γ-globulin 5 g daily for 3 days; M, mouth; mPSL, methylprednisolone 0·5–1 g daily for 3 days; NSAID, nonsteroidal anti-inflammatory drug; PSL, prednisolone.

Bioscience, Tokyo, Japan) and the 3-amino-9-ethylcarbazole (AEC) substrate kit (Nichirei Bioscience).

Paraffin-embedded skin sections were dewaxed and rehydrated. After antigen retrieval and blocking of nonspecific binding sites, sections were incubated with a monoclonal antibody against CD16 (Lab Vision, Fremont, CA, U.S.A.). Immunostaining was then carried out using Histofine Simple Stain AP (M) (Nichirei Bioscience) and a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate kit (Dako Japan). After a second round of endogenous peroxidase inhibition and blocking of nonspecific binding sites, sections were incubated with a monoclonal antibody (CD45RO; Nichirei Bioscience) specifically recognizing mature T cells. Immunostaining was developed using Histofine Simple Stain MAX PO (M) and the AEC substrate kit.

Evaluation method

The degree of epidermal damage was classified according to the graft-versus-host reaction (GVHR) grades described by Lerner *et al.*,[15] as follows: grade 0, normal skin; grade I, mild vacuolization of epidermal cells with occasional dyskeratotic bodies; grade II, diffuse vacuolization of basal cells with scattered dyskeratotic bodies; grade III, subepidermal cleft formation; grade IV, complete epidermal separation. In this study, skin sections that showed dermal infiltration without epidermal change were classified as grade 0.

The number of CD16+ or CD45RO+ cells was evaluated by counting the total number of positive cells along a linear 500-μm section of the epidermis. Three or four typical lesions were examined, and mean cell counts were obtained by calculating the average number of positive cells.

The grade of epidermal damage, number of cells and immunofluorescence stains were blindly evaluated by two of the investigators (M.T. and S.M.). Significant variations in the results were not observed.

Statistical analysis

Statistical significance was determined using Student's paired t-tests. Differences were considered statistically significant at $P < 0{\cdot}05$.

Results

CDI4+ CD16+ cells infiltrate in the epidermis and the dermoepidermal junction of Stevens–Johnson syndrome/toxic epidermal necrolysis lesions

Consistent with previous reports, CD14+ cells were observed in the epidermis, at the dermoepidermal junction, and in the upper dermis of skin biopsies from all 11 patients with SJS/TEN. Interestingly, > 80% of the CD14+ cells coexpressed CD16 (Fig. 1B) whereas > 90% of the CD16+ cells expressed CD14 (Table 2). CD16 is typically expressed not only by CD14+ cells, but also by natural killer cells. In the SJS/TEN skin lesions examined in this study, a small number of CD16+ cells coexpressed CD2 but not CD56 (data not shown).

To examine the characteristics of CD14+ CD16+ cells further, expression of CD11c, HLA-DR and CD163 was examined (Table 2). CD11c, a marker for monocytes and dendritic cells (DCs), was expressed in almost all CD16+ cells located in the epidermis and at the dermoepidermal junction (Fig. 1Ca). HLA-DR expression was observed in CD16+ cells and in other cells, including keratinocytes (Fig. 1Cb). CD163, a macrophage marker, was weakly expressed on CD16+ cells at the dermoepidermal junction and in the upper dermis (Fig. 1Cc).

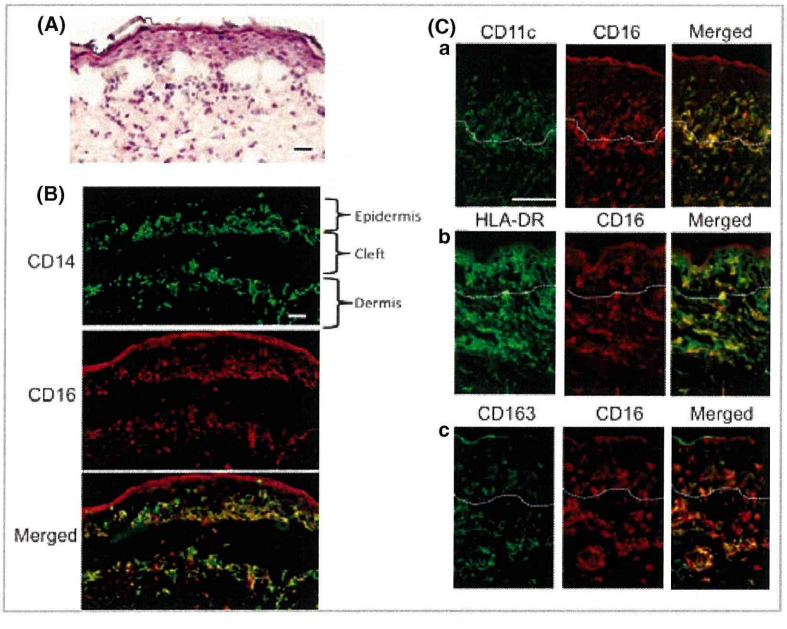

Fig 1. Immunostaining of monocyte, dendritic cell and macrophage markers in skin biopsies. (A) Haematoxylin and eosin staining in cryosection of patient 2 with toxic epidermal necrolysis (TEN). (B) Costaining of CD16 (red) and CD14 (green) in cryosections of skin lesions from patient 2 with TEN. (C) Costaining of CD16 (red) and CD11c, HLA-DR and CD163 (green) in cryosections of skin lesions from patients with Stevens–Johnson syndrome (patients 6, 5 and 9, respectively). Dotted line indicates the dermoepidermal junction. Black or white bar = 100 μm.

Table 2 Expression of markers in CD16+ cells infiltrating in the epidermis and at the dermoepidermal junction of Stevens–Johnson syndrome/toxic epidermal necrolysis

Patient	CD14	CD11c	HLA-DR	CD163	CD80	CD86	CD137 ligand
1	+++	+++	+++	(+++)	+++	+++	++
2	+++	+++	+++	(+++)	+++	+++	+++
3	+++	+++	+++	+++	+	++	+++
4	+++	+++	+++	+++	−	+	+++
5	+++	+++	+++	(++)	+++	+	+++
6	+++	+++	+++	(++)	+++	+++	+++
7	+++	+++	+++	(+)	+	+	+++
8	+++	+++	+++	(+++)	++	++	+++
9	+++	+++	+++	(+)	+	+	+++
10	+++	+++	+++	+	−	+	++
11	+++	+++	+++	+++	−	+	+

−, completely negative; +, positive in < 50% cells; ++, positive in > 50% and < 90% cells; +++, positive in > 90% cells; (+), weakly positive in < 50% cells; (++), weakly positive in > 50% and < 90% cells; (+++), weakly positive in > 90% cells.

However, in patients 3, 4, 10 and 11, in whom skin biopsies were performed later than in the other patients, strong CD163 expression was noted (Table 2). These findings indicated that the CD14+ CD16+ cells were of monocyte lineage, and that CD163 expression increased with time after disease onset.

The presence of a smaller number of CD14+ CD16+ cells was also observed in the lower dermis. These cells expressed either no or lower levels of CD11c, but higher levels of CD163, properties typical of macrophages (Fig. 1Ca, c).

CD16+ cells express CD80, CD86 and CD137 ligand

Monocyte lineage cells, such as macrophages and DCs, activate T cells through engagement of the T-cell receptor with a cognate peptide–MHC complex and an additional costimulatory signal, such as CD80, CD86 or CD137L. We examined whether CD16+ cells also express these costimulatory factors. CD80 was expressed on CD16+ cells in eight of the 11 SJS/TEN skin samples, and CD86 on CD16+ cells in all samples, albeit to varying degrees (Fig. 2A, B and Table 2).

CD137L is an important costimulatory factor for activating CD8+ T cells.[13,14] We found that CD137L was strongly expressed on CD16+ cells in the SJS/TEN skin samples (Fig. 2C, Table 2). The expression of CD137, a receptor for CD137L, was revealed on CD8+ T cells infiltrating the lesion skin (Fig. 2D). The CD137L-expressing cells were seen bound to CD8+ T cells expressing CD137 in the dermis (Fig. 2E).

CD16+ cells appear at the dermoepidermal junction in the very early phase of Stevens–Johnson syndrome/toxic epidermal necrolysis

Infiltration of CD16+ cells may be an early event in the pathogenesis of SJS/TEN, indicating that CD16+ cells do not accumulate as a result of epidermal damage, but instead precede it. This scenario was confirmed in an evaluation of skin samples from the normal-appearing skin of eight patients with SJS/TEN. Samples were taken from sites near skin lesions, before the development of epidermal detachment, and paraffin embedded for immunohistological analysis. The results showed a small number of CD16-expressing cells at the dermoepidermal junction and/or in the dermis in five of the eight samples (Fig. 3A). The skin lesions of three of these five patients (Fig. 3Aa, b, d) had progressed to epidermal detachment, despite intensive therapy. Moreover, epidermal detachment developed at the sites where samples were obtained from normal-appearing skin.

Detailed information for patient 6 (Table 1 and Fig. 3Aa) is shown in Figure 3B. Skin biopsies were obtained from an erythematous lesion and from a nonlesional area on the patient's abdomen (Fig. 3Ba). The lesion expanded rapidly, and normal-appearing skin had progressed to epidermal detachment 2 days postbiopsy. Within the lesion, numerous apoptotic keratinocytes, subepidermal blister formation, and mononuclear cell infiltration were observed (Fig. 3Bb). Normal-appearing nonlesional skin showed no sign of epidermal damage and the infiltration of only a small number of mononuclear cells into the dermis (Fig. 3Bc). A slight separation was noted at the dermoepidermal junction. In cryosections of the nonlesional skin, immunostaining revealed HLA-DR expression in epidermal keratinocytes and in the dermal component (Fig. 3Bd). Moreover, it was readily apparent that CD16+ cells had already appeared at the dermoepidermal junction (Fig. 3Be). CD16+ cells expressing CD137L (data not shown) were seen bound to CD137-expressing cells at the dermoepidermal junction (Fig. 3Bf). These findings suggest that CD16+ cells are involved in the development of epidermal damage.

Correlation between the number of CD16+ cells and epidermal damage

Mild epidermal damage is often observed in drug rashes other than SJS/TEN. In EM or MP, there is a slight degree of epidermal damage, such as scattered apoptotic keratinocytes and/or liquefaction degeneration of basal cell layer. We assessed the relationship between the degree of epidermal damage and the number of CD16+ cells in 47 patients with drug rash, including those with SJS, TEN, MP and EM. Because the grade of epidermal damage varies according to the timing and location of the skin biopsy, in this study the degree of epidermal damage in all skin samples was classified according to GVHR grade. Thus, MP/EM samples were classified as grade 0 or I, except for one case of grade II. In contrast, all cases of SJS/TEN were classified as grades II–IV. In grade 0 skin samples, no CD16+ or CD45RO+ cells were observed in the epidermis, similar to normal skin (Fig. 4Aa). In grade I, no or only a few CD16+ cells had infiltrated the dermoepidermal junction, as was the case in MP and EM (Fig. 4Ab). In the diseases with higher GVHD grades, the number of CD16+ cells increased in parallel with the degree of epidermal damage

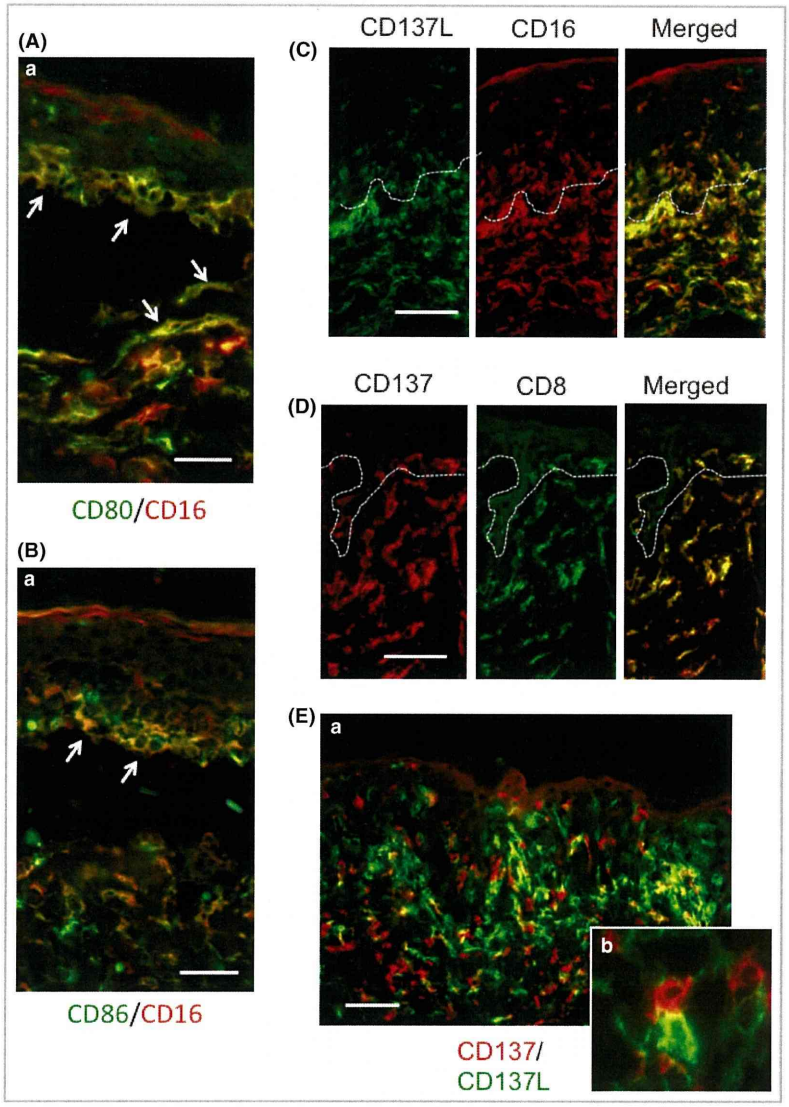

Fig 2. CD80, CD86 and CD137 ligand (CD137L) expression on CD16+ cells. (A) Costaining of CD16 (red) and CD80 (green) in cryosections of skin lesions from patient 2 with toxic epidermal necrolysis (TEN). (B) Costaining of CD16 (red) and CD86 (green) in cryosections of skin lesions from patient 1 with TEN. Arrows indicate cells coexpressing CD16 and CD80/CD86. (C) Costaining of CD16 (red) and CD137L (green) in cryosections of skin lesions from patient 6 with Stevens–Johnson syndrome (SJS). (D) Costaining of CD137 (red) and CD8 (green) in cryosections of skin lesions from patient 5 with SJS. (E) Costaining of CD137 (red) and CD137L (green) in cryosections of skin lesions from patient 9 with SJS (a). CD137-expressing cells (red) are bound to CD137L-expressing cells (green) (patient 7) (b). Dotted lines indicate the dermoepidermal junction. White bar = 100 μm.

(Fig. 4Ac, d, e). The number of CD16+ or CD45RO+ cells that infiltrated the epidermis, including the dermoepidermal junction, was counted and the result compared according to the respective grade. The number of CD16+ cells increased significantly with increasing grade, while there was no difference in the number of CD45RO+ cells among grades I–IV (Fig. 4B).

Discussion

The present study focused on the numerous CD14+ CD16+ monocyte lineage cells that infiltrate the epidermis and upper dermis of SJS/TEN skin lesions. Abundant infiltration of monocytes in the epidermis of TEN skin lesions was demonstrated in earlier studies using immunohistochemical analysis.[9–11] On the other hand, recent studies have examined cells collected from the bullous fluid of patients with TEN. Monocytes were found to be a minor component, especially in the early phase of the disease.[7] Thus, there is a discrepancy between the cellular composition of TEN lesions observed by histological examination vs. that determined using flow cytometric analysis of bullous fluid cells. Although the reason for this difference is unclear, morphological changes occurring in monocytes after stimulation may make it difficult to collect these cells from blister fluid.

In peripheral blood, CD14+ CD16+ monocytes are a minor component, accounting for approximately 10% of monocytes.[12] These cells are referred to as proinflammatory monocytes due to their production of higher levels of TNF-α and lower levels of interleukin-10 after stimulation.[16,17] Additionally, these cells express CD11c and HLA-DR, and have been recognized as circulating DC precursors.[18,19] In our study, CD14+ CD16+ cells in the skin lesions of patients with SJS/TEN expressed CD11c and HLA-DR, similar to CD14+ CD16+ monocytes in blood. This finding provides new insight into the role of monocytes in SJS/TEN lesions.

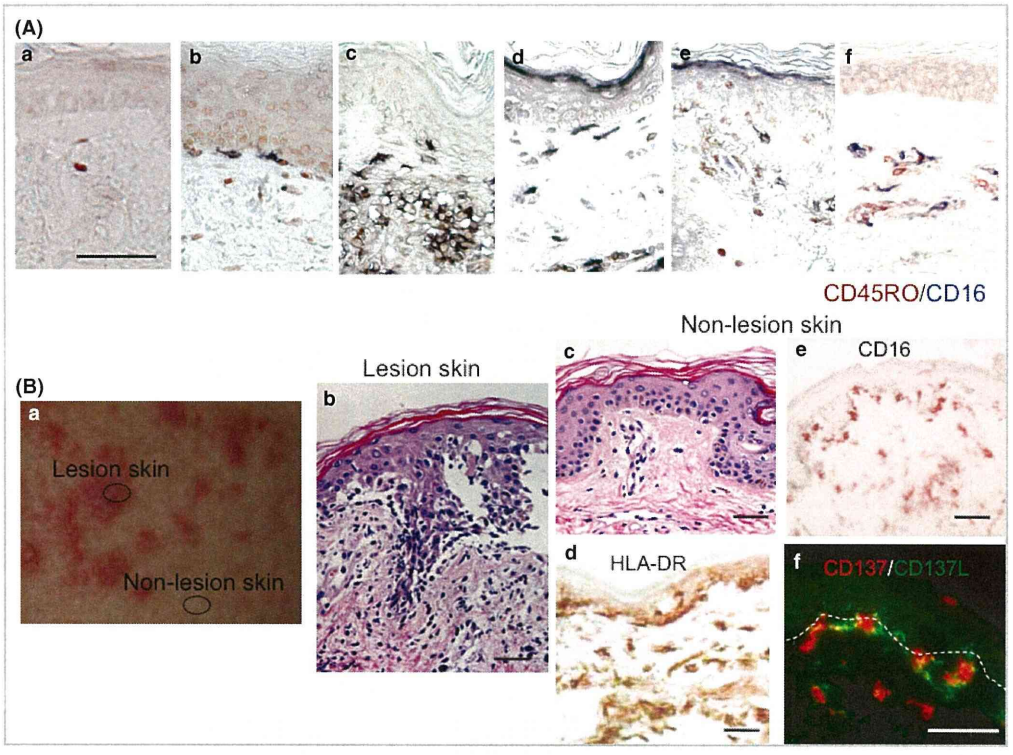

Fig 3. The very early phase of toxic epidermal necrolysis (TEN). (A) Immunostaining for CD16 and CD45RO in paraffin-embedded skin from normal skin (a) and the normal-appearing skin of four patients with Stevens–Johnson syndrome/TEN (b–f). (B) Abdominal skin biopsies were taken from an area of macular erythema and the adjacent nonlesional skin (a). Haematoxylin and eosin staining of the erythematous skin lesion (b) and the nonlesional skin (c). Immunostaining for HLA-DR (d) and CD16 (e) in a cryosection from nonlesional skin. CD137-expressing cells bind to CD137 ligand (CD137L)-expressing cells at the dermoepidermal junction (f). Dotted line indicates the dermoepidermal junction. Black or white bar = 100 μm.

CD14+ CD16+ cells in the epidermis and at the dermoepidermal junction of SJS/TEN lesions were found to express costimulatory ligands, such as CD80, CD86 and CD137L. CD80/CD86 and CD137L activate T cells through their receptors, CD28 or CD137, respectively, in a process involving engagement of the T-cell receptor and a cognate peptide–MHC complex.[13,20] CD80–CD28 and CD86–CD28 are widely considered to be the most important costimulatory pathways for CD4+ T-cell activation and expansion,[21–23] while CD137L–CD137 signalling plays an important role in the expansion of CD8+ T cells.[13,24,25] CD137, a member of the TNF receptor family, is absent from resting T cells but is rapidly expressed on antigenic stimulation.[26–28] CD137 signalling could avoid the activation-induced cell death of CD8+ T cells and prolong their proliferation.[29–32] Even if CD8+ T cells become anergic after stimulation, CD137L could reactivate these T cells to proliferate.[32] Moreover, CD137 signalling directly augments the cytotoxic function of CD8+ T cells,[33–37] with costimulation by CD137L increasing perforin and granzyme A expression.[25] Thus, it is conceivable that activation of the CD137L–CD137 system enhances CD8+ T cell-mediated immune responses. In fact, the systemic administration of agonistic anti-CD137 or endogenous CD137L has been shown to cause a potent cell-mediated immune response against tumours[38–42] or an enhanced virus-specific CD8+ T-cell response, in the latter case resulting in rapid viral clearance.[26,43–46] Together, these observations provide evidence for the importance of the CD137L–CD137 system in CD14+ CD16+ cells and for a key role of CD8+ T cells in the skin in the development of SJS/TEN.

In contrast to CD137L expression levels, which did not differ widely among SJS/TEN samples, CD80 and/or CD86 expression by CD16+ cells tended to be stronger in the early than in the late phase of SJS/TEN. In particular, strong CD80 and CD86 expression was observed in the skin of patients in whom the area of epidermal detachment progressed after the biopsy was performed and treatment was started (patients 1, 2, 5 and 6). Costimulation with CD80/CD86 and CD137L has been reported to promote the proliferation and cytotoxicity of CD8+ T cells more strongly than CD137L alone.[32] Thus, CD80 and CD86 expression levels may be involved in enhancing the activity of CD8+ T cells and thereby contribute to disease progression. However, because this study was based on only a few cases, further research is necessary.

Our results also demonstrated that the number of CD16+ cells infiltrating the epidermis and dermoepidermal junction

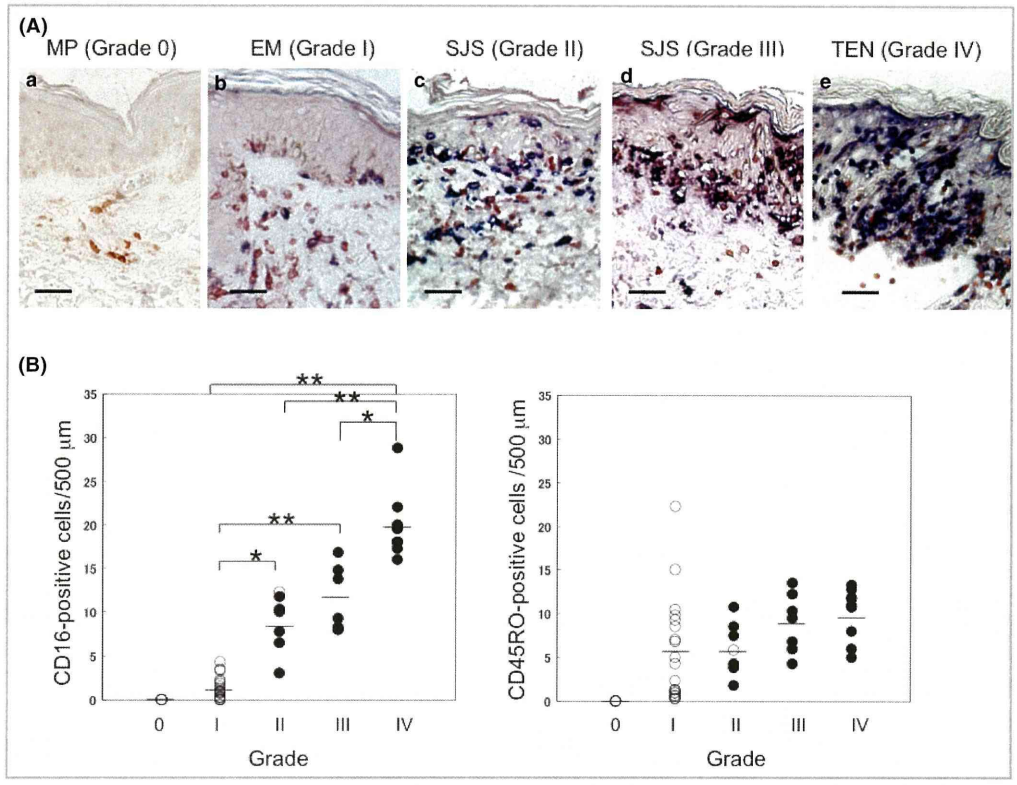

Fig 4. Correlation between CD16+ or CD45RO+ cell number and histopathological graft-versus-host reaction grade of epidermal damage. (A) Immunostaining of CD16 (blue) and CD45RO (red) in skin biopsies. Skin biopsy from patients with grade 0 maculopapular-type drug rash (MP) (a), grade I erythema multiforme-type drug rash (EM) (b), grade II and grade III Stevens-Johnson syndrome (SJS) (c and d, respectively), and grade IV toxic epidermal necrolysis (TEN) (e). Black bar = 100 μm. (B) CD16+ and CD45RO+ cells in the epidermis and at the dermoepidermal junction were counted in skin sections prepared from patients with MP/EM (open circles) and SJS/TEN (closed circles). *$P < 0{\cdot}05$; **$P < 0{\cdot}001$.

correlated with the grade of epidermal damage. Because the number of CD16+ cells was higher than that of CD45RO-expressing T cells in grade IV disease, it seems likely that CD16+ cells not only enhance CD8+ T-cell activity but also contribute directly to the epidermal damage. Cytotoxic effects of CD16+ monocytes against mesangial cells or tumour cells have been demonstrated in *in vitro* studies.[17,47] Because CD16+ monocytes produce abundant TNF-α,[16,17] cytokines may, at least in part, be responsible for the epidermal damage. Significant epidermal damage due to combined TNF-α and IFN-γ signalling was demonstrated in a skin explant model developed for the analysis of acute graft-versus-host disease following bone marrow transplantation.[48] In another study, peripheral blood mononuclear cells (PBMC) from bone marrow transplant donors were cultured with the recipient's irradiated lymphocytes and then cocultured with skin explants obtained from the recipient. Significant epidermal damage, including apoptosis of epidermal keratinocytes, resembling that in drug rash or SJS/TEN was observed.[49,50] This phenomenon could be reproduced in cell-free supernatants from allostimulated PBMC, and the epidermal damage inhibited by the addition of anti-TNF-α or anti-IFN-γ antibody.[50] TNF-α and IFN-γ levels in the supernatants correlated with the severity of cellular damage in the epidermis.[50] The infiltration of CD14+ CD16+ cells of monocyte lineage into the epidermis along with CD8+ T cells causes TNF-α levels to increase, which may lead to the induction of apoptosis in keratinocytes in response to IFN-γ produced by T cells, leading to epidermal damage. Clinically, the efficacy of anti-TNF-α therapy in SJS/TEN treatment has been reported,[51,52] but the direct cytotoxicity of CD14+ CD16+ cells of monocyte lineage against keratinocytes remains to be investigated.

In conclusion, our data suggest that CD14+ CD16+ monocyte lineage cells contribute to the epidermal damage characteristic of SJS/TEN. These cells may enhance the proliferation and cytotoxicity of CD8+ T cells in addition to prolonging their activation in SJS/TEN via CD137, causing severe epidermal damage. Thus, inhibition of the interaction between CD14+ CD16+ cells and CD8+ T cells may be a useful strategy in the treatment of SJS/TEN. Blockage of the CD137/CD137L system is also expected to reduce the cytotoxicity of CD8+ T cells. A previous report demonstrated that, in the mouse, blockage of the CD137–CD137L pathway reduced the number and cytotoxicity of CD8+ T cells and ameliorated the acute GVHR.[35,53] The mechanism underlying the accumulation and function of CD14+ CD16+ monocyte lineage cells in SJS/TEN remains to be elucidated.

What's already known about this topic?

- In the skin lesions of patients with Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), monocytes are abundantly observed in the epidermis and at the dermoepidermal junction.

What does this study add?

- CD14+ monocytes in the lesional skin of SJS/TEN coexpress CD16, CD11c and HLA-DR, similar to proinflammatory monocytes or dendritic cell precursors in blood.
- These cells may enhance the cytotoxicity of CD8+ T cells through expression of CD137 ligand, CD80 and CD86.
- These cells might be implicated directly in the epidermal damage, because the number of CD16+ cells infiltrating the epidermis increases depending on the grade of epidermal damage.

Acknowledgments

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INVITED ARTICLE

New aspects of drug-induced hypersensitivity syndrome

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ABSTRACT

Drug-induced hypersensitivity syndrome (DIHS) is caused by a limited number of specific drugs and is characterized by late onset, infectious mononucleosis-like symptoms, and herpesvirus 6 (HHV-6) reactivation. Recently, the involvement of herpes viruses other than HHV-6, such as Epstein–Barr virus and cytomegalovirus, has been reported. Many approaches have been used to analyze the pathological mechanism, and have revealed new aspects of DIHS. Here, we focused on three key recent findings regarding DIHS: (i) overlap between DIHS and Stevens–Johnson syndrome/toxic epidermal necrolysis; (ii) the relevance of Epstein–Barr virus in the development of infectious mononucleosis-like symptoms of DIHS; and (iii) roles of monomyeloid precursors increased in the blood and plasmacytoid dendritic cells increased in the lesion skin in HHV-6 reactivation.

Key words: drug-induced hypersensitivity syndrome, Epstein–Barr virus, herpesvirus 6, Stevens–Johnson syndrome, toxic epidermal necrolysis.

INTRODUCTION

Drug-induced hypersensitivity syndrome (DIHS) has been established as a clinical entity in severe cutaneous drug adverse reactions (Table 1).[1,2] DIHS is characterized by a limited number of causative drugs, late onset, clinical similarity to infectious mononucleosis-like syndrome and prolonged clinical course due to relapse.[1,2]

Anticonvulsants, such as carbamazepine, phenytoin, phenobarbital and zonisamide, are major culprit drugs of DIHS, and lamotrigine has been reported to be a new causative drug of DIHS in Japan.[1,2] In addition to anticonvulsants, allopurinol, diaphenylsulfone (DDS), salazosulfapyridine and mexiletine can also cause DIHS.[1,2] Although minocycline-induced DIHS is rare in Japan, it has been reported frequently in other countries.

Drug-induced hypersensitivity syndrome typically develops 2–6 weeks after the initiation of drug administration, and the initial symptoms are fever and maculopapular eruptions that may progress to erythroderma. Lymphadenopathy, hepatitis, renal dysfunction and hematological abnormalities, such as leukocytosis, eosinophilia and atypical lymphocytosis, are observed to varying degrees. Flare-ups involving clinical signs, such as fever, eruption or hepatitis, often occur several weeks after withdrawal of the causative drug.[2,3]

In DIHS, human herpesvirus (HHV)-6 is commonly reactivated 2–3 weeks after onset, sometimes resulting in relapse of fever and hepatitis.[3] The reactivation of herpesviruses other than HHV-6, including cytomegalovirus (CMV), Epstein–Barr virus (EBV) and HHV-7, has also been reported.[4–6] In general, the cascade of virus reactivation initiated by HHV-6 and HHV-7 extends to EBV and CMV. EBV and HHV-7 reactivation are thought to have no clinical relevance. In contrast, when CMV reactivation is observed, recurring transient fever, skin rash, or severe

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Table 1. Diagnostic criteria for drug-induced hypersensitivity syndrome (DIHS) established by a Japanese consensus group

1. Maculopapular rash developing >3 weeks after starting with a limited number of drugs
2. Prolonged clinical symptoms 2 weeks after discontinuation of the causative drug
3. Fever (>38°C)
4. Liver abnormalities* (alanine aminotransferase >100 U/L)
5. Leukocyte abnormalities (at least one present)
   a. Leukocytosis (>11 × 10^9/L)
   b. Atypical lymphocytosis (>5%)
   c. Eosinophilia (>1.5 × 10^9/L)
6. Lymphadenopathy
7. Human herpesvirus 6 reactivation

The diagnosis is confirmed by the presence of the seven criteria above (typical DIHS) or of the five (1–5) (atypical DIHS). *This can be replaced by other organ involvement, such as renal involvement.

complications, such as myocarditis, pneumonia or gastrointestinal bleeding, may occur.[7,8]

The clinical features of DIHS have been established over the past 10 years, and the pathological mechanism has been analyzed. This review discusses recent insights into three topics: (i) overlap between DIHS and Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN); (ii) relevance of EBV in the development of infectious mononucleosis-like symptoms of DIHS; and (iii) the roles of monomyeloid precursors increased in the blood and plasmacytoid dendritic cells increased in the lesion skin in HHV-6 reactivation.

OVERLAP BETWEEN DIHS AND SJS/TEN

Drug-induced hypersensitivity syndrome is distinct from other severe cutaneous drug reactions, such as SJS and TEN. However, DIHS cases associated with skin manifestations similar to SJS/TEN have been reported.[9,10] They fulfilled all or most of the criteria of DIHS, and coincidently revealed mucosal involvement and epidermal detachment (Fig. 1). It seems likely that these findings caused confusion in the definition of DIHS.

However, specific types of skin rash are not essential for diagnosis of DIHS, because DIHS is diagnosed based on its characteristic clinical course, multiple organ involvement and detection of herpesvirus reactivation. The skin manifestations of DIHS include maculopapular rash, erythema multiforme, exfoliative dermatitis, acute generalized exanthematous pustular dermatosis-like eruption or erythroderma. Mucosal involvement may also be observed in some cases to a lesser extent. On the other hand, SJS/TEN is diagnosed by characteristic skin and mucosal

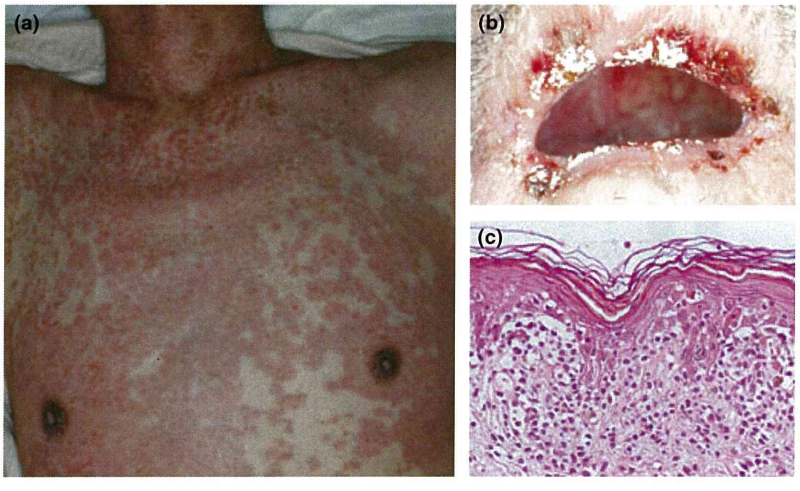

Figure 1. Drug-induced hypersensitivity syndrome (DIHS) due to zonisamide associated with Stevens–Johnson syndrome. DIHS developed after administration of zonisamide for 3 months. High fever, leukocytosis, appearance of atypical lymphocytes, hepatitis and lymphadenopathy were observed. Human herpesvirus 6 and cytomegalovirus reactivation were confirmed. Atypical target lesions appeared over the whole body (a), and oral and genital mucosae were involved (b). The area of epidermal detachment was approximately 1%. Histopathologically, numerous apoptotic cells were observed in the epidermis (c) (hematoxylin–eosin, original magnification ×100).

manifestations, but not by organ involvement. Therefore, a case diagnosed as SJS or TEN based on epidermal detachment may also be diagnosed as DIHS based on the clinical course, laboratory findings and viral reactivation.

Late onset is a characteristic feature of DIHS. In contrast, SJS/TEN most often begins in the early stages, after the initiation of causative drugs.[11] We compared the day of onset between SJS/TEN, DIHS and SJS/TEN–DIHS overlapping cases. Data from 46 cases of SJS/TEN, 167 of DIHS and seven overlapping cases due to anticonvulsants (carbamazepine, phenobarbital, phenytoin, and zonisamide) were collected. These cases were published as articles or meeting abstracts in medical journals between 2000 and 2009 in Japan, and the available data included the time between the start of drug administration and onset of symptoms. The onset of SJS/TEN was within 3 weeks after the start of drug administration in 67% of cases (Fig. 2). In contrast, DIHS developed at 2–6 weeks in 80% of cases, and occurred most frequently at 4–5 weeks. Thus, the day of onset was clearly different between anticonvulsant-induced DIHS and SJS/TEN. Moreover, six of seven overlapping cases developed symptoms at 4–5 weeks after commencement of drug administration. These findings indicated that overlapping SJS/TEN–DIHS is a subtype of DIHS.

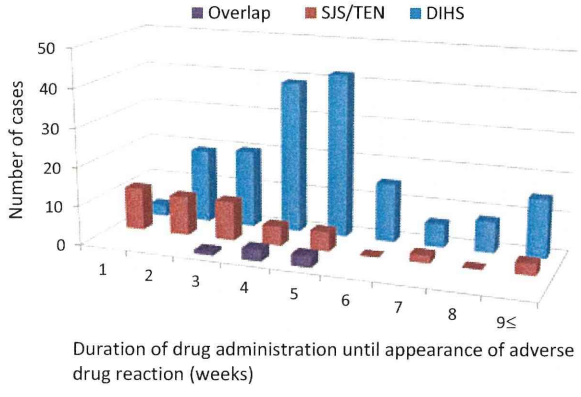

Figure 2. Duration of drug administration until the appearance of drug adverse reaction. Japanese cases of carbamazepine-induced Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), drug-induced hypersensitivity syndrome (DIHS), and SJS/TEN–DIHS overlapping were analyzed.

INFECTIOUS MONONUCLEOSIS-LIKE SYMPTOMS AND VIRAL INFECTION

Infectious mononucleosis-like symptoms, including lymphadenopathy, hepatitis, renal dysfunction, atypical lymphocytosis and mononucleosis, are observed during the early phase of DIHS. Interestingly, HHV-6 reactivation is observed 2–3 weeks after onset, but not concomitant with infectious mononucleosis-like symptoms. HHV-6 DNA can be detected after the appearance of infectious mononucleosis-like symptoms, followed by an increase in anti-HHV-6 antibody titer (Fig. 3). These findings suggest that HHV-6 reactivation may not participate directly in production of the infectious mononucleosis-like symptoms.

Recently, Picard *et al.*[12] suggested that EBV participates in the development of multi-organ involvement with drug rash and eosinophilia with systemic symptoms (DRESS). Although DRESS and DIHS are often mistakenly taken to be synonymous, they are not identical,[1,13] and DIHS patients probably represent severe cases of DRESS. That is, DRESS consists of DIHS and other mild drug rashes. Therefore, HHV-6 reactivation is detected in the majority of DIHS patients but in only a limited number of DRESS patients. Picard *et al.*[12] analyzed HHV-6, HHV-7, CMV and EBV reactivation by polymerase chain reaction (PCR) in peripheral blood mononuclear cells (PBMC) and serum from 40 DRESS patients, and detected HHV-6 reactivation in 17 of 38 cases (45%). EBV DNA was detected in the PBMC from all of these patients, and EBV reactivation occurred in 16 of 38 cases (42%). The authors demonstrated that expanded CD8 T cells during the course of DRESS

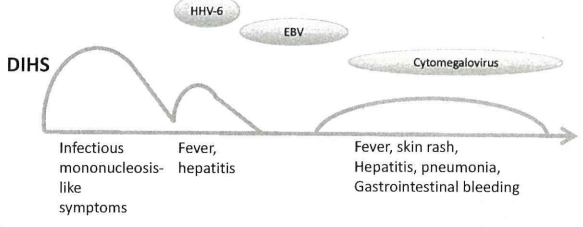

Figure 3. Relationship between infectious mononucleosis-like symptoms and viral reactivation in drug-induced hypersensitivity syndrome (DIHS). Infectious mononucleosis-like symptoms precede human herpesvirus (HHV)-6 reactivation, followed by Epstein–Barr virus (EBV) and cytomegalovirus reactivation.

responded to EBV antigen, regardless of whether EBV reactivation was detected. No healthy controls showed the reactivity. Moreover, culprit drugs induced EBV reactivation in EBV-transformed blood B cells from patients in a dose-dependent manner. From these results, they proposed that culprit drugs induce EBV reactivation and antigenic presentation may trigger a multi-organ immune response through activation of CD8 T cells.

It has been suggested that reactivation of EBV usually occurs after HHV-6 reactivation (Fig. 3).[4,5] We examined viral DNA in whole blood from 32 DIHS patients in whom HHV-6 reactivation was confirmed during the clinical course. EBV DNA was detected in 24 of these 32 patients (75%) (Mikiko Tohyama, unpubl. data, 2009). High viral loads were detected in approximately half of these patients, but associated symptoms were not confirmed. EBV DNA could not be detected up to day 12 of the disease, and was detected for the first time on day 13. The highest copy number of EBV DNA was observed with or after HHV-6 reactivation in 22 of 24 patients positive for EBV DNA. In infectious mononucleosis caused by primary EBV infection, the viral DNA is abundant in the early phase of the disease and decreases in association with recovery from the acute phase.[14,15] During reactivation, the antiviral immune response differs from that of primary infection. Therefore, the viral load may also be different from that of primary infection. EBV that is disseminated to multiple organs before onset of DIHS may be cleared rapidly by cytotoxic T cells. The virus may then proliferate again, following a decline of immune activation. Therefore, it will be necessary to investigate EBV in the very early phase of DIHS. However, we were unable to examine EBV DNA in whole blood collected within 3 days after onset in the present study. Picard *et al.*[12] observed HHV-6 or EBV reactivation on day 0 in 14 patients. However, day 0 was the day on which patients were included in the study and not the day of onset.

Although CMV was not detected by PCR in the study of Picard *et al.*,[12] we detected CMV DNA in blood samples from 10 of 32 patients (31%) (Mikiko Tohyama, unpubl. data, 2009). CMV reactivation is a common feature in severe DIHS in Japan, and the viral DNA was detected in the late phase.[4–6,8] As the viral load of CMV in DIHS is similar to that of EBV, CMV may also play an important role in DIHS, similar to EBV. In fact, Hashizume *et al.*[16] reported that CD8 T cells bearing T-cell receptor (TCR)-Vβ recognizing CMV-derived antigenic peptides were expanded during the infectious mononucleosis-like clinical course in a Japanese DIHS patient.

Viral infection is an attractive hypothesis to explain the infectious mononucleosis-like symptoms of DIHS. Further studies are required to determine viral involvement in the very early phase of DIHS and to characterize expanded CD8 T cells in these patients. It is not sufficient to examine EBV alone, and future studies should include other viruses, such as CMV.

INVOLVEMENT OF SKIN INFLAMMATION IN HHV-6 REACTIVATION

As mentioned above, HHV-6 viremia is detected after the occurrence of infectious mononucleosis-like symptoms in DIHS. It remains unclear how HHV-6 is reactivated in DIHS. As HHV-6 is often detected in the saliva, it is possible that HHV-6 reactivation always occurs in salivary glands.[17,18] In addition, under conditions of immunosuppression, HHV-6 may be reactivated from latently infected monocytes.

Recently, Hashizume *et al.*[19] reported that the numbers of monomyeloid precursors were increased in the peripheral blood of DIHS patients. These cells were not observed in control subjects. The population of monomyeloid precursors increased within 10 days after onset of disease and then decreased gradually thereafter. It is of interest that HHV-6 viral antigen was detected in the cells from 8–30 days, but not in a patient in whom HHV-6 reactivation was not detected. These observations suggest that the monomyeloid precursors appearing early in the peripheral blood may participate in HHV-6 reactivation of DIHS.

Human herpesvirus 6 viremia is detected in graft-versus-host disease (GVHD), measles and dengue fever, as well as DIHS.[20–24] HHV-6 reactivation in GVHD is frequently associated with skin rash.[21,22] Measles and dengue fever show clinical features similar to DIHS, such as skin rash, liver dysfunction, lymphadenopathy and the appearance of atypical lymphocytes. It seems likely that the presence of cutaneous inflammation is essential for HHV-6 reactivation. Immune reaction accompanied with skin inflammation may cause an increase in the number of

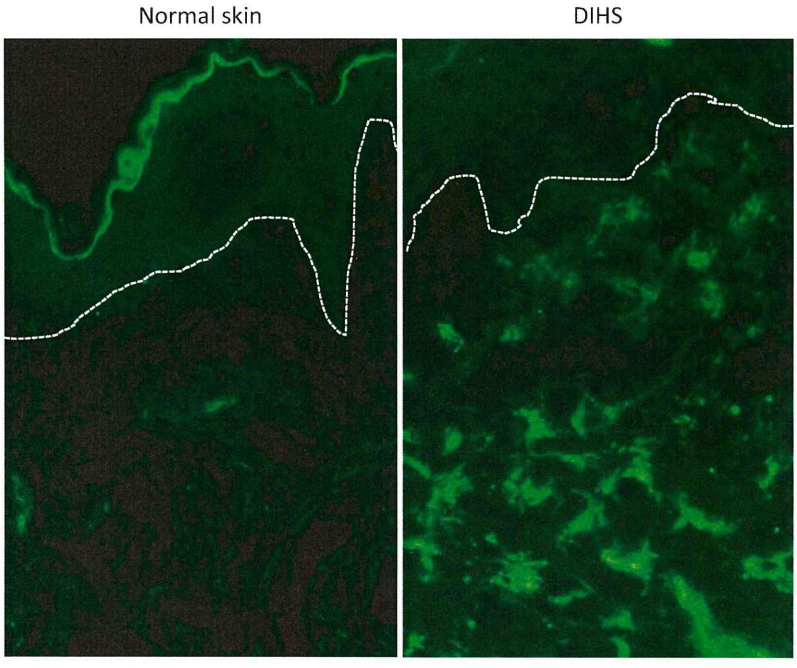

Figure 4. Infiltration of plasmacytoid dendritic cells (pDC) in normal skin (left) and lesion skin of drug-induced hypersensitivity syndrome (DIHS) (right). The lesion skin was obtained from DIHS due to carbamazepine on day 8 of the disease. In this patient, human herpesvirus 6 reactivation was observed 5 days later. Numerous CD123-positive cells (labeled green), indicating pDC, were found to have infiltrated into the dermis (original magnification ×200).

monomyeloid precursors and facilitate HHV-6 reactivation. Alternatively, monomyeloid precursors may be recruited into the lesion skin, where HHV-6 reactivation and proliferation may occur. As monomyeloid precursors express cutaneous lymphocyte-associated antigen, it is possible that these cells infiltrate into the skin.[19]

Human herpesvirus 6 reactivation is considered to require immunosuppression. Shiohara's group has reported several immune defects, such as a marked decrease in serum immunoglobulin (Ig)G levels and circulating B-cell number at the onset stage, as well as dysfunction of regulatory T cells.[25,26] Skin inflammation may be involved in the induction of immunosuppressive conditions. More recently, Sugita *et al.*[27] demonstrated a reduction in the number of plasmacytoid dendritic cells (pDC) in the peripheral blood of DIHS patients, but an increase in these cells in the lesion skin (Fig. 4). Human pDC are a subset of leukocytes capable of producing large amounts of interferon (IFN)-α, which induces maturation of B cells to produce IgG and thus plays a crucial role in antiviral responses.[28,29] Therefore, they proposed that pDC in the circulation may accumulate in the skin and thus reduce the number of pDC in the circulation. Therefore, antiviral responses may be reduced, facilitating viral reactivation in peripheral blood and tissues other than the skin. However, further studies are required to determine whether the lack of pDC in the circulation reduces the antiviral defense activity, thus leading to viral reactivation.

CONCLUSION

Drug-induced hypersensitivity syndrome has been established as a clinical entity in severe cutaneous drug adverse reaction in which HHV-6 reactivation is a hallmark. Many approaches, such as epidemiology, virology and immunology, have been used to analyze the pathological mechanism of DIHS. However, various questions still remain unsolved. Further studies are required to investigate the roles of herpesvirus, including HHV-6, EBV and cytomegalovirus in this disease entity.

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