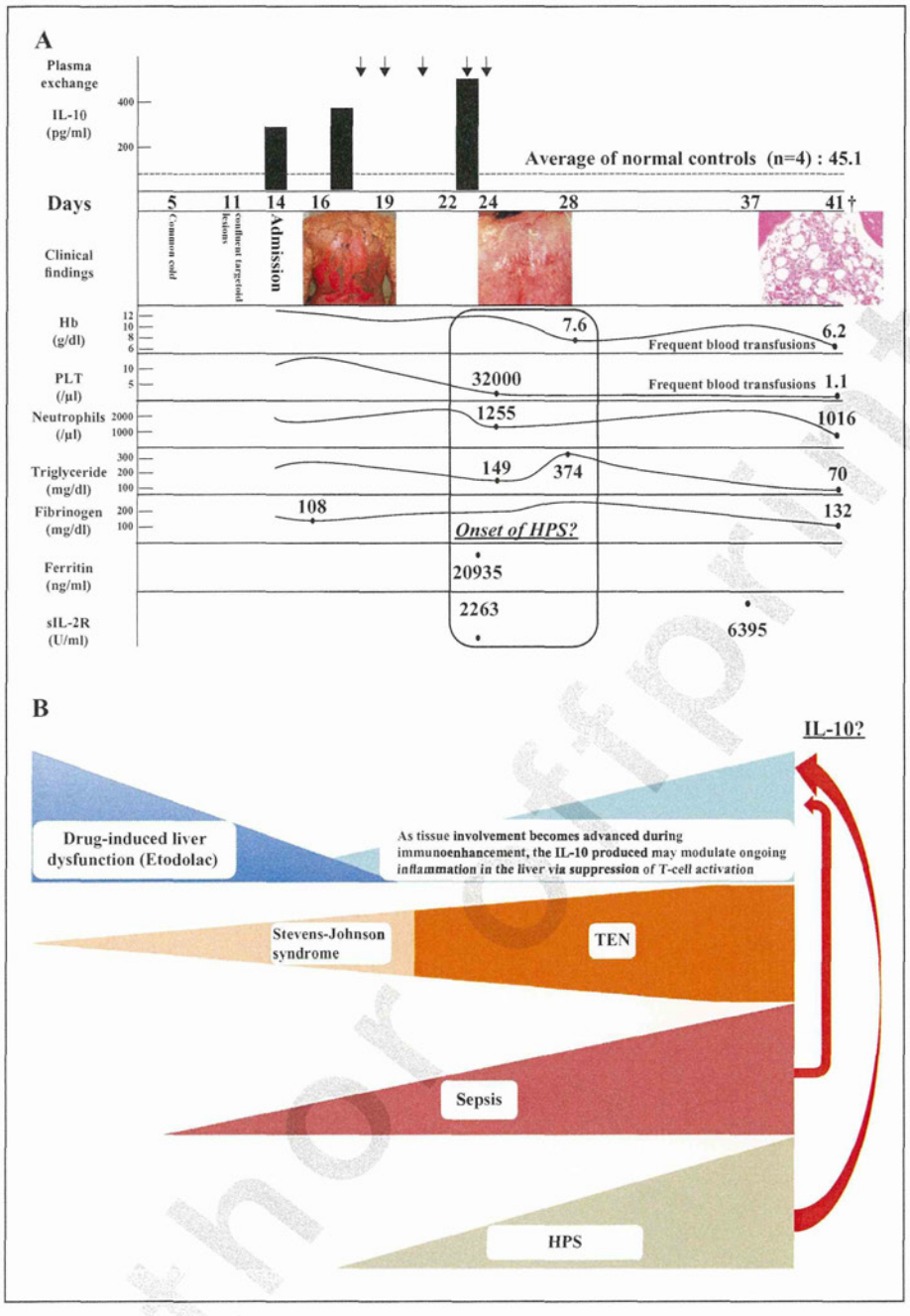

Figure 1. A) History, time course, and clinical findings of a patient with toxic epidermal necrolysis. Serum levels of IL-10 (measured by ELISA; Becton-Dickinson) and other indicators over the course of hospitalisation are shown. The average levels of IL-10 in normal control subjects are indicated by the broken line. The small inserts show the extensive skin involvement in the patient, and a photomicrograph of a section of involved skin indicates epidermal necrosis and eosinophil infiltration. Serum IL-10 levels were apparently further elevated after plasma exchange (indicated by arrows at the top of the figure). The proposed period of onset of haemophagocytic syndrome is indicated by the central boxed region. Abbreviations and symbols used: Hb, haemoglobin; PLT, platelet count; sIL-2R, soluble IL-2 receptor; HPS, haemophagocytic syndrome; †, death of the patient. **B)** Hypothesis relating prolonged elevated serum IL-10 levels to progression of liver dysfunction. Although drug-induced liver damage may improve after discontinuation of nonsteroidal immunosuppressant treatment, the elevated IL-10 production caused by sepsis and haemophagocytic syndrome is proposed to modulate ongoing liver inflammation via suppression of T-cell activation.

elevation of IL-10 production. Our observations are consistent with IL-10 being a key regulating factor in TEN progression. ■

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High-dose Intravenous Immunoglobulin Monotherapy for Drug-induced Hypersensitivity Syndrome

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Drug-induced hypersensitivity syndrome (DIHS), also known as drug rash with eosinophilia and systemic symptoms (DRESS), is a severe, multi-organ, adverse reaction characterized by erythema with facial oedema, fever, lymphadenopathy, hepatitis, and leukocytosis with eosinophilia (1–3). In more than 60% of DIHS cases, human herpesvirus (HHV)-6 reactivation is observed and is associated with an unfavourable outcome (4). High-dose corticosteroid therapy is a controversial treatment for DIHS/DRESS because patients may become susceptible to infections (5). We describe here the case of a DIHS/DRESS patient successfully treated with high-dose intravenous immunoglobulin (IVIG) containing a high titre of anti-HHV-6 antibodies.

CASE REPORT

A 49-year-old woman had developed a postoperative infection after radical resection of a pelvic chondrosarcoma. Methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Serratia marcescens* were identified in the surgical wound by bacterial culture. Although various antibiotics were sequentially administered, the infection persisted and her body temperature remained higher than 38.5°C for approximately 3 months. She also experienced neuropathic pain in her right leg due to a nerve injury and was treated with a 3-month course of mexiletine. The drug was discontinued when the neuralgia abated. Three days later, erythema spread over her body and facial oedema appeared. Physical examination revealed cervical and inguinal lymphadenopathy. Maculopapules, with follicular accentuations, were distributed over her body, apart from the areas around the eyes and mouth (Fig. 1A and B). There was no enanthema in her oral cavity. Laboratory investigations showed an elevated white blood cell count (10,400/μl, normal range: 3,600–9,200/μl) with eosinophilia (17%, normal: <5%) and atypical lymphocyte (1%), elevated liver enzymes (aspartate aminotransferase, 125 IU/l, normal: 11–30 IU/l; alanine transaminase, 107 IU/l, normal: 5–42 IU/l), C-reactive protein (2.4 mg/dl, normal: <0.1 mg/dl) and serum anti-human herpesvirus (HHV)-6 IgG titre (1:640), and decreased serum IgG (840 mg/dl, normal: 870–1,700 mg/dl). No increase was observed in the titre of antibodies against Epstein-Barr virus or cytomegalovirus antigens. Skin histology showed mild liquefaction degeneration, a few dyskeratotic cells in the epidermis, and perivascular lymphocytic infiltration in the dermis (Fig. 2). Some lymphocytes were large, with condensed nuclei, resembling lymphoma cells. We diagnosed the patient with "atypical" DIHS based on the diagnostic criteria for DIHS by a Japanese consensus group (the patient's score was 5 points) (3). Patch testing and lymphocyte transformation test with mexiletine after the resolution gave negative results. However, we considered it as the causative drug because there have been accumulating cases of DIHS/DRESS induced by this agent, and the 3-month administration in this case was consistent with the incubation time for this disease. We started IVIG 3 days after the eruption appeared. To avoid any worsening of the pre-existing infection, the patient was treated with high-dose IVIG therapy at 0.4 g/kg/day for 5 successive days instead of the more "conventional" high-dosage corticosteroid therapy. We used batches containing relatively high titres of IgG antibodies against HHV-6 (1:160, as assessed by the fluorescent antibody method) and cytomegalovirus (86.4 U/ml, as assessed by enzyme immunoassay). The body temperature returned to normal in 3 days and the eruption had disappeared completely 7 days after treatment was initiated. The elevated liver enzymes returned to normal levels thereafter. There was no worsening of the surgical wound infection after treatment. The titres of herpes viruses after IVIG are shown in Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1168).

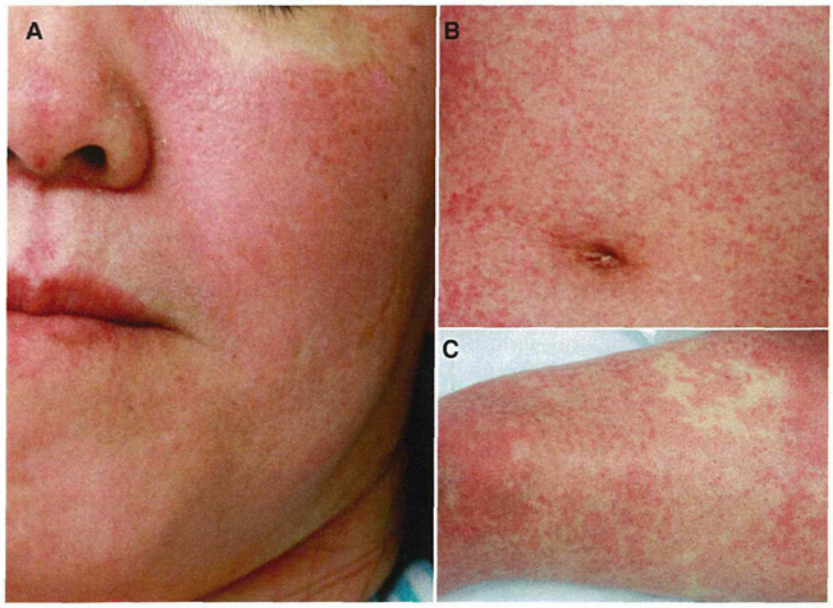

Fig. 1. (A) Maculopapules, sparing the eyes and mouth. Erythema on (B) the trunk and (C) the thigh.

DISCUSSION

Anti-inflammatory agents are usually used for the treatment of severe adverse drug reactions (SADRs) such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and DIHS/DRESS, since the simple discontinuation of the culprit drugs does not reduce the inflammatory response in such cases. High-dose corticosteroid therapy has been used empirically in SADRs. However, serious conditions affecting patients with SADRs may

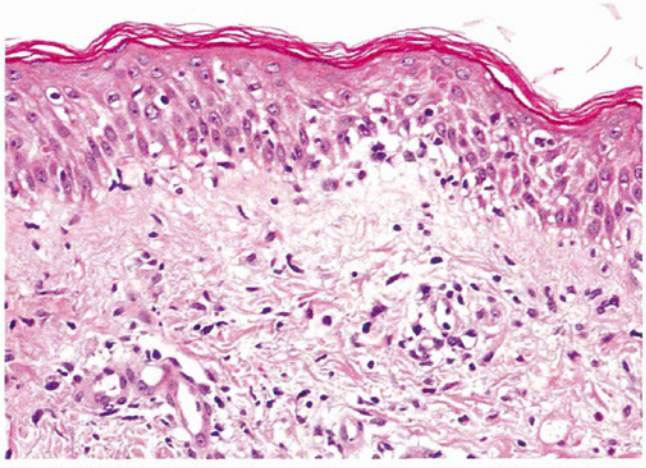

Fig. 2. Mild liquefaction degeneration, a few dyskeratotic cells in the epidermis and perivascular lymphocytic infiltration of the dermis were observed. Haematoxylin and eosin ×200.

impose restrictions on the choice of treatment. The present case was induced by mexiletine, one of the more "notorious" culprits, and showed characteristic features of DIHS/DRESS (2–4). However, we hesitated to use high-dosage corticosteroid therapy because the patient also had a severe post-operative infection. We therefore decided to use high-dose IVIG monotherapy to treat this case, although only a few cases of such treatment have been reported (6, 7). This course of treatment rapidly reduced disease activity without causing any worsening of the wound infection. We successfully treated this patient with high-dose IVIG monotherapy.

DIHS is distinct from other SADRs because of the dynamic alterations of immunity observed during the course of the disease. The phenotype of circulating lymphocytes is altered from a CD4+ cell-dominant profile to CD8+ cell-dominant profile that coincides with the time of viral reactivation (8). Regulatory T cells in the circulation and skin initially increase in number, but decrease further thereafter, in parallel with the observed functional impairment (9). The concentration of serum immunoglobulins also decreases during the early phase of DIHS/DRESS (10). These unique immunological phenomena may be associated with immunological defects that cause HHV-6 reactivation, a unique event in DIHS/DRESS, although the precise mechanism remains unknown.

High-dose IVIG therapy for DIHS/DRESS has two immunological effects. First, it compensates for the decreased immunoglobulin concentration and immunological defects for protection of HHV-6 infection. Batches containing high titres of antibodies against the viruses might be particularly effective in preventing HHV-6 reactivation (11). Secondly, high-dose IVIG has a substantial anti-inflammatory effect that regulates the immune response, as seen in the treatment of autoimmune disorders (12). Our experience in this case should encourage clinicians to consider IVIG as a therapeutic option for DIHS/DRESS patients, especially in those patients with a high risk of infection.

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LETTER TO THE EDITOR

Deep venous thrombosis associated with cytomegalovirus reactivation in drug-induced hypersensitivity syndrome

Editor

Drug-induced hypersensitivity syndrome (DIHS) is a severe drug allergy characterized by damage to multiple extracutaneous organs and frequent reactivation of human herpesviruses (HHVs) during the disease course.[1] This is the first case of simultaneous emergence of deep venous thrombosis (DVT) and CMV reactivation in a DIHS patient.

A 78-year-old male patient with an 8-day history of recurrent fever, general fatigue and nausea was transferred to our hospital. Physical examination revealed fever (38.6°C) and respiratory distress. At the time of admission, the anti-CMV IgG was 6.4 (ELISA, normal, <2), and the anti-CMV IgM was 0.45 (normal, <0.8). Chest roentgenography and computed tomography demonstrated bilateral interstitial shadows in the bilateral lung fields, suggestive of atypical pneumonitis. Minocycline was started. Twenty-two days after admission, AST and ALT suddenly increased to 114 and 139 U/L respectively. Four days later, high fever suddenly developed with simultaneous emergence of facial oedematous erythema and a maculopapular rash (Fig. 1a). A remarkable increase in white blood cells (16 800/μL) was observed with increased neutrophils (15 080/μL), atypical lymphocytosis (2%) and monocytosis (660/μL). Skin biopsy of the abdominal lesion disclosed mild lymphocytic infiltration into the epidermis and adnexal appendages with focal necrotic keratinocytes (Fig. 1b). Based on the criteria of the Japanese consensus group, diagnosis of 'possible' DIHS was made.[1] Thus, minocycline was discontinued. Administration of prednisolone at 50 mg/day relieved skin manifestations; the prednisolone was gradually reduced to 10 mg/day for 3 weeks thereafter without recurrence. On the sixth day of administration of prednisolone 10 mg/day, his lower legs swelled acutely, and erythematous macules developed again over the trunk and extremities with severe facial oedema and high fever (>38.8°C), suggestive of a flare up of symptoms in DIHS.[1] Laboratory data revealed a high C-reactive protein level (7.93 mg/dL) and increased AST and ALT levels (44 and 59 U/L respectively). In addition to these findings, we observed increased levels of D-dimers (7.3 μg/mL; normal, <1 μg/dL). Neither anti-cardiolipin antibody nor lupus anticoagulant was detected. Computed tomography revealed a large thrombosis from the inferior caval vein to bilateral iliac veins and popliteal veins (Fig. 1c and 1d) as a concomitant medical problem. At that time, we detected seroconversion of CMV IgG and IgM (52.6 and 3.68 respectively), although no cells positive for CMV antigen pp65 was observed. Anticoagulant therapy with heparin was initiated while continuing prednisolone at

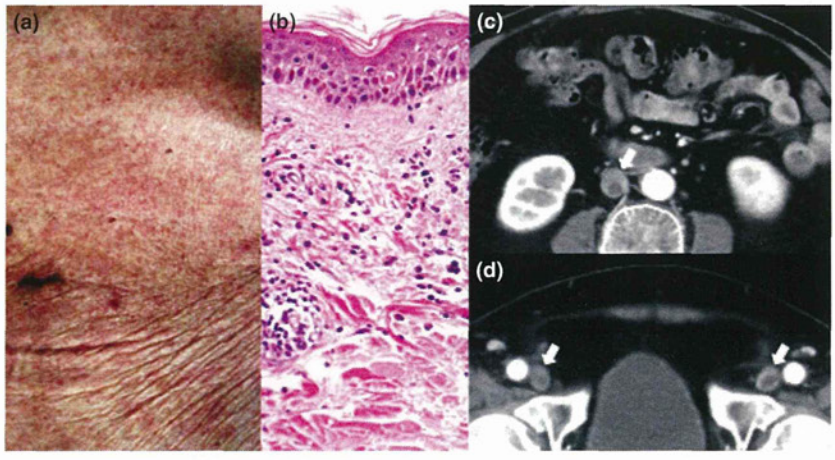

Figure 1 Clinical and pathological features of the present case. Skin biopsy of the maculopapular rash spreading on the abdominal skin (a) reveals lymphocytic infiltration into the epidermis and appendages of the skin (b) (HE staining, original ×400). During the course, thromboses were found in the inferior caval vein (c) and bilateral femoral veins (d). Arrows indicate thrombosed veins on contrast-enhanced computed tomography images.

10 mg/day. AST and ALT gradually returned to normal levels in 2 weeks. The rash disappeared with resolution of leg swelling without medication for CMV infection.

We found that CMV seroconversion was chronologically associated with development of DVT in this case. In spite of accumulating cases of DIHS with CMV reactivation and its complications,[2–5] there has been no case of DVT during DIHS, suggesting that it might have been underestimated. The presence of risk factors plays a prominent role in assessing the pretest probability of DVT: acute infection, older age (>75 years), burden cancers and previous history of thrombosis.[6] In this case, although the patient was aged, no previous history of burden cancers or thrombosis enhanced a crucial contribution of CMV to development of DVT. Two recent case–control studies proved that CMV infection was independently associated with thrombosis among a whole cohort.[7,8] This may occur because of the effect of a procoagulant on endothelial cells[9] or induction of anti-phospholipid antibody that binds to the molecules, mimicking CMV antigens.[10]

We should draw attention to development of DVT as a serious complication of DIHS in addition to well-known serious sequels by CMV reactivation, such as encephalitis, carditis, gastroenteritis and pneumonia.

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Review

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Recent progress of elucidating the mechanisms of drug hypersensitivity

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Recent technical approaches to investigating drug hypersensitivity have provided a great deal of information to solve the mechanisms that remain poorly understood. First, immunological investigations and *in silico* analysis have revealed that a novel interaction between T cells and antigen-presenting cells, namely the pharmacological interaction concept, is involved in drug recognition and the hapten theory. Second, progress in immunology has provided a new concept of CD4+ T cell subsets. Th17 cells have proven to be a critical player in acute generalized exanthematous pustulosis. Our recent findings suggest that this subset might contribute to the pathogenesis of Stevens-Johnson syndrome/toxic epidermal necrolysis. Third, alarmins, molecules associated with innate immunity, are also associated with exaggeration and the persistence of severe drug hypersensitivity. The latest innovative techniques are providing a new landscape to examine drug hypersensitivity.

Key words: Allergy; Drug hypersensitivity; Human herpesvirus-6; Innate immunity; T cell; T cell receptor

INTRODUCTION

Why does drug eruption occur? Why does inflammation aggravate even if administration of the culprit drug is stopped? Unfortunately, we have no answers to these questions. However, the recent technical approach of the gene expression analysis has provided a great deal of information to solve them. Genome-wide analysis has revealed that individuals with certain human leukocyte antigen (HLA) polymorphisms have a 10 to more than 1,000-fold risk of drug hypersensitivity [1-4]. It will provide us with an order-made treatment to prevent severe drug hypersensitivity. Furthermore, recent *in silico* analysis makes it possible to reveal exquisite interactions between HLA molecules and drugs in the drug-antigen recognition of T cells [5], thereby providing us with a new perspective for drug discovery without hypersensitivity. In addition, recent studies using gene expression analysis have enabled the search for aggravation factors of severe drug hypersensitivity [6], which provides us with a novel therapeutic strategy. The latest innovative techniques are providing a new landscape to examine drug hypersensitivity.

Mode of recognition of drug molecules in drug hypersensitivity

Because most drugs are small molecules, their covalent

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binding to self-derived proteins, such as membrane proteins and serum albumin, is necessary for immunogenicity. Essentially, a new epitope is presented by covalent binding of drugs with self-proteins, which is recognized by T cells as a haptenic antigen. It has been accepted as an important event for small molecules to be immunogenic to elicit contact dermatitis in experimental models [7], and may be central to the mechanism of drug allergies (Fig. 1A) [8]. However, it has been recently demonstrated that a high affinity of the drug for major histocompatibility complex (MHC) expressed on antigen-presenting cells (APCs), or for T cell receptors (TCRs) expressed on T cells, increases the possibility of activating T cells (Figs. 1B and C) [8]. Such binding is non-covalent with electrical or van der Waals forces, and can be easily dissociated by mechanical force. This type of interaction between T cells and APCs in drug recognition has been proposed as the pharmacological interaction concept (*p-i* concept) by Pichler et al. [9]. Interestingly, in several previous reports, anticonvulsant-specific T cell clones tend to have Vβ 5.1, despite the diversity of HLA alleles [10-13]. Under such conditions, antigen processing appears unnecessary for T cell activation because T cells activate even in the presence of APCs fixed with glutaraldehyde and the culprit drug [12]. Some clones usually do not require HLA matching of APCs for the response to the drug, indicating non-MHC restricted T cell recognition [14]. Such observations are accounted for by the "*p-i* concept" binding between the drug and TCR. On the other hand, in Han Chinese, individuals with HLA-B*1502 have a more than 2,500-fold risk of carbamazepine hypersensitivity [4]. Recent computer analyses of the molecular structure revealed an exquisite interaction between two molecules in the drug-antigen recognition. In such cases, the drug non-covalently binds to HLA-B 15:02, which is indicative of the "*p-i* concept" style binding [5].

In the hapten theory, after sensitization with the haptenized drug antigen, memory T cells recognize the antigenic epitope presented by APCs, with the arms of the TCR to critically fit its structure. However, the unique drug recognition manner in the *p-i* concept does not require the sensitization of T cells to the drug [9]. Basically, memory T cells that express TCRs with a certain affinity to the drug activate even if they are not sensitized to the drug, in the presence of the drug and APCs. Japanese individuals with HLA-A*3101 have a greater risk of carbamazepine hypersensitivity [3] in addition to Han Chinese with HLA-B*1502 [4]. Such cases show no correlation between this HLA polymorphism and the severity of drug hypersensitivity, suggesting that other factor(s) may contribute to exacerbate drug hypersensitivity. The size of the TCR repertoire of memory T cells is influenced by the individual's previous infections. The larger the size of the TCR repertoire of memory T cells, the higher the possibility of drug affinity for TCRs. Therefore, the number of T cells that bind a drug in the manner of the "*p-i* concept" may be increased in individuals who have experienced more infections. This scenario is well fitted with our clinical observations, although further investigation is needed to address this issue.

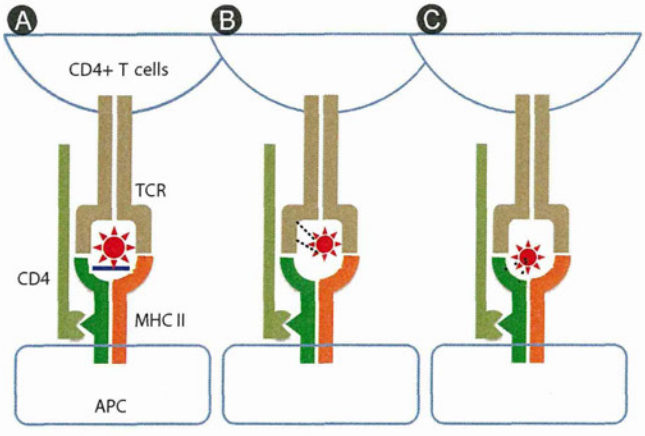

Fig. 1. Interaction of T cells and antigen-presenting cells (APCs) in drug antigen recognition. A new epitope is presented by covalent binding of drugs with self-proteins, which is recognized by T cells as a haptenic antigen (A). On the other hand, a high affinity of the drug for T cell receptors (TCRs) expressed on T cells (B), or major histocompatibility complex (MHC) expressed on APCs (C), increases the possibility of activating T cells (*p-i* concept, modified in the Fig. of ref. [9]).

Characterization of drug-specific T cells

Effector T cells are divided into CD4+ and CD8+ subtypes, and the former is classified into Th0, Th1, Th2, Th9, Th17 and Th22, depending on the profile of released cytokines (Fig. 2) [15]. The quality and quantity of activated T cells reflects the clinical skin manifestations of drug hypersensitivity. The severity of CD8+ T cell infiltration into the skin is correlated with the degree of epidermal cell necrosis, which determines the clinical phenotype such as maculopapular eruption, Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) (Fig. 3) [12]. In SJS/TEN, CD8+ T cells in blisters greatly activate to gain a natural killer (NK) cell marker, CD94/NKG2c, on their cell surface and acquire an antigen-independent killing activity resembling that of NK cells via binding to classical MHC (HLA-E) expressed on inflammatory epidermal cells [16]. Therefore, the activation status of CD8+ T cells in skin and blood is a predisposing factor for severe drug hypersensitivity such as SJS/TEN. On the other hand, a Th2-shifted immune response

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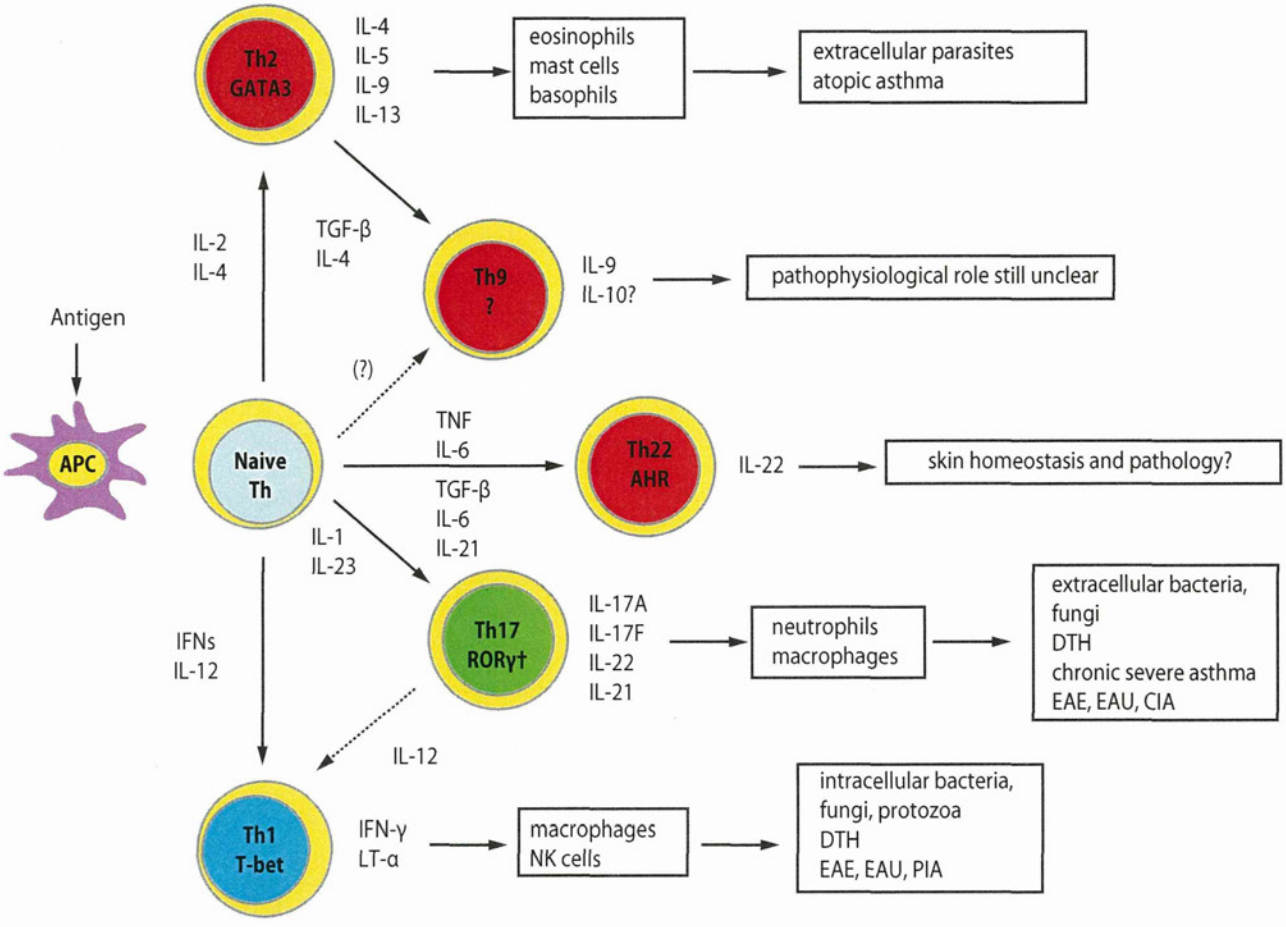

Fig. 2. Functional subsets of effector CD4+ T cells [15]. APC, antigen-presenting cell; GATA3, the transcription factor GATA-binding protein-3; IFN, interferon; TGF, transforming growth factor; AHR, aryl hydrocarbon receptor; RORγt, retinoic acid-related orphan receptor γt; T-bet, T-box expressed in T cells; DTH, delayed type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; EAU, experimental autoimmune uveitis; CIA, collagen-induced arthritis; PIA, peptoglycan-induced arthritis.

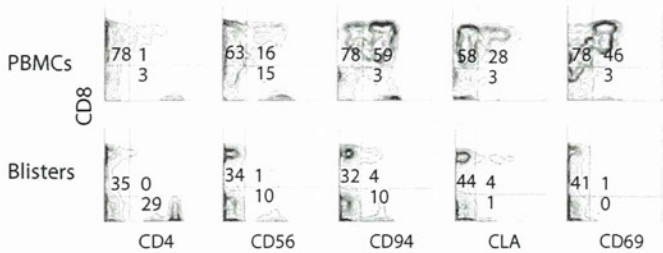

Fig. 3. Phenotypic analysis of peripheral blood mononuclear cells (PBMCs) and mononuclear cells in blisters (Blisters) in a toxic epidermal necrolysis patient. Numbers indicate percentages of total cells.

induces tissue eosinophilia and overproduction of IgE, resulting in the emergence of edematous erythema and wheals. β-lactam-specific T cells tend to produce Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13, which is indicative of Th2 cells and consistent with the clinical presentations [17]. IL-17-producing T cells, known as Th17 cells, are attracting attention as pathognomonic T cells in psoriasis, which also produce IL-8 (CXCL-8), a chemokine for neutrophils [18]. High serum levels of IL-17 and IL-8 are observed in acute generalized exanthematous pustulosis that clinically resembles pustular psoriasis, suggesting a contribution of Th17 to the pathology [19, 20]. Th17 cells cooperatively exaggerate the inflammatory responses of Th1/Tc1 [18]. Interestingly, CD4+ T cells with the ability to produce high levels of IL-17 are found among the drug-specific CD4+ T cell clones established from SJS/TEN patients (Fig. 4, unpublished data). Damaged epidermal cells in SJS/TEN release prostaglandin E2 and alarmins, which promotes differentiation and proliferation of Th17 cells activated by IL-23-producing dendritic cells after signaling via their receptors. This observation may be one of the explanations for the persistence of

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these serious diseases.

Regulatory T cells (Tregs) are another modifier of the inflammatory responses in drug hypersensitivity, which comprise natural and induced CD4+CD25+Foxp3+ Tregs and other cell types. Because of functional impairment of CD4+CD25+Foxp3+ Tregs [21-26], the risk of drug hypersensitivity would tend to rise in autoimmune disorders [27]. Anti-CCR4 antibody treatment is a novel therapy for adult T cell leukemia lymphoma (ATL/L) to kill CCR4-expressing ATL/L cells, while it may also reduce the number of CD4+CD25+Foxp+ Tregs that express CCR4. During such treatment, drug eruptions including SJS/TEN develop in around 70% of cases, suggesting that impairment of Tregs is a high risk factor for drug hypersensitivity. Functional impairment of Tregs has been found in drug-induced hypersensitivity syndrome (DIHS), which may be associated with a prolonged disease course [27].

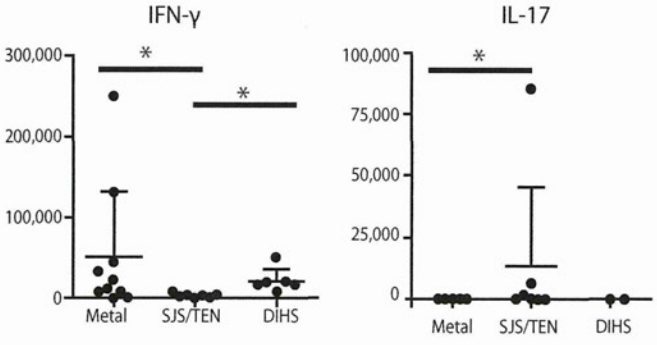

Fig. 4. IFN-γ and IL-17 productions of drug-specific T cell clones (TCCs) after stimulation with anti-CD3 antibody for 3 days. TCCs were established from patients with gold and nickel allergies (Metal), SJS/TEN and DIHS. Y-axis, cytokine concentrations (pg/mL). $^{*}p < 0.05$, Dunn's Multiple Comparison Test. SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis; DIHS, drug-induced hypersensitivity syndrome.

Innate immunity and alarmins

Oppenheim proposed that damage associated molecular pattern molecules (DAMPs) released from damaged cells are cues for initiating immune responses in various organs by their activation after interacting with pattern recognition receptors and/or toll-like receptors [28, 29]. These molecules promote rapid recruitment of bone marrow-derived leukocytes to target tissues for inflammation and regeneration under various aseptic inflammatory conditions including SJS and TEN [6]. This molecular

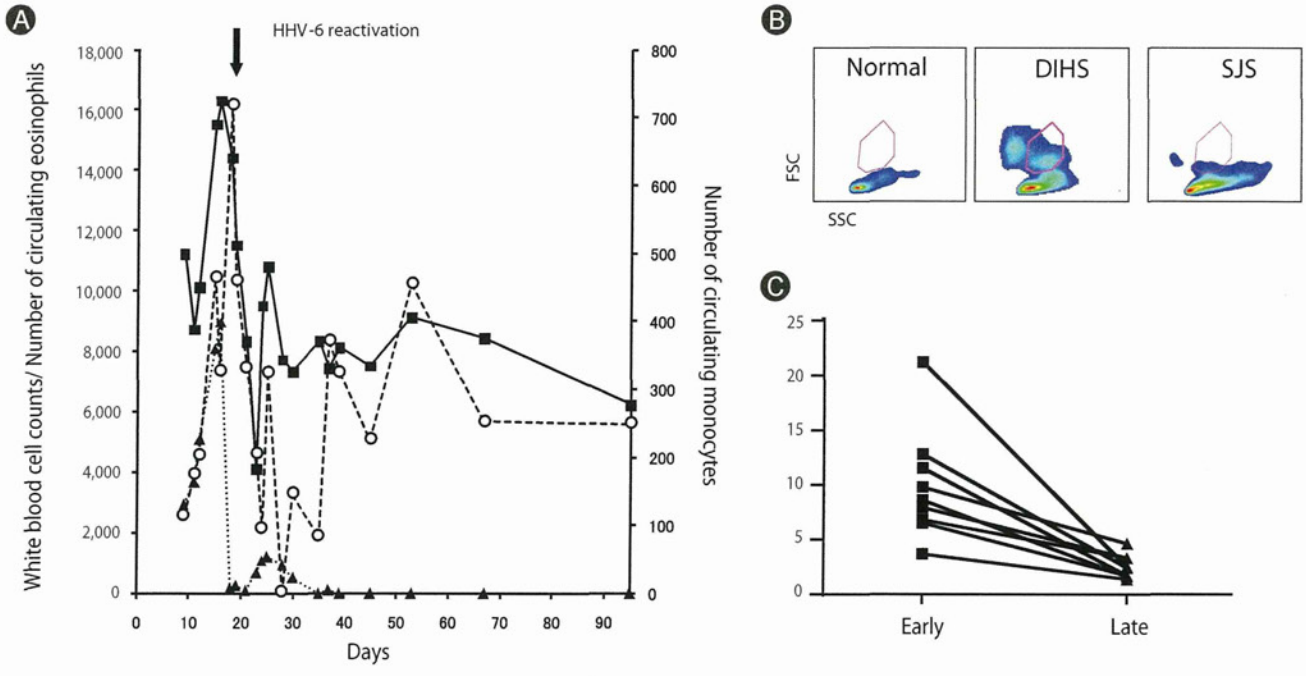

Fig. 5. Circulating monocytes in drug-induced hypersensitivity syndrome (DIHS) patients. (A) Representative data of total blood cell counts (closed squares), numbers of eosinophils (closed triangles) and monocytes (open circles). Numbers indicate cell counts (/mm^3). An arrow indicates human herpes virus (HHV)-6 reactivation. (B) Flowcytometric analysis of peripheral blood mononuclear cells in normal individuals and patients with DIHS and Stevens-Johnson syndrome (SJS). Monocytes from DIHS patients show higher side scatter counts (SCC). FSC, forward scatter counts. (C) Percentage of monocytes at early (≤21 days after the onset) and late (>21 days after the onset) phase.

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group contains various substances from simple chemicals, such as uric acid, to larger molecules such as IL-33 and DNA. Endogenous DAMPs are now designated as alarmins. High mobility group box-1 (HMGB-1), one of the most well-known alarmin members, is a non-histone protein with dual functions, namely transcriptional regulation by loose binding to chromatin inside cells and a cue for inflammation outside cells, with high potency to attract and activate various immunocompetent cells including monocytes and myeloid cells [28]. Recently, high expression levels of HMGB-1 have been found in blood from SJS patients [30]. Granulysin is a member of the saposin-like protein family, which is classified into 9- and 15-kDa isoforms. The 9-kDa granulysin is a well-described pore forming cytotoxic molecule with proinflammatory activity, and is considered as a crucial molecule to induce apoptosis of keratinocytes in SJS/TEN [31]. The 15-kDa granulysin has been recently accepted as an alarmin, which activates monocytes and dendritic cells via binding the toll-like receptor-4/Myd88, rather than a cytotoxic molecule [32, 33]. In addition, 15-kDa granulysin may act as an alarmin in SJS/TEN to promote Th17 cell responses by activation of dendritic cells.

Mechanisms of human herpes virus (HHV) reactivation in DIHS

The most mysterious phenomenon in DIHS is reactivation of various HHV types, including cytomegalovirus, Epstein-Barr virus, HHV-6 and HHV-7, during the disease course [34, 35]. HHV-6 reactivation has been found in more than 60% of such cases in association with unfavorable outcomes [36]. Our concern is why HHV-6 frequently reactivates in DIHS. We found that the number of circulating monocytes transiently increase (>500/mm^3) within 3 weeks and return to normal levels thereafter (Figs. 5A and C). The monocytes transiently observed in DIHS are different from conventional monocytes in terms of side scatter counts (SSC) and phenotype [37]. Monocytes from DIHS patients show higher SCC (Fig. 5B), suggesting enriched organelles, and comprise a minor $CD14^{high}CD16^{-}$ population and a major $CD14^{low}CD16^{+}$ population that express skin-associated molecules such as CCR4, CLA markedly and CCR10 partially (manuscript in preparation). This observation implies that they are a precursor mono/myeloid subset that differentiates into skin-resident macrophages. We also found the HHV-6 genome and virus structures in some of these monocytes (manuscript in preparation). Because some mono-myeloid cells are latently infected with HHV-6 as virus reservoirs [38], monocytes that transiently circulate in DIHS patients appear to have originated from such cells. We found close contacts between monocytes and T cells in the skin, and the presence of HHV-6 in skin-resident $CD4^+$ T cells (manuscript in preparation). HHV-6 infection of $CD4^+$ T cells is an indispensable event for virus replication and reactivation [39]. Monocytes may lead this critical event in $CD4^+$ T cells, suggesting that HHV-6 infects $CD4^+$ T cells in the skin of DIHS patients. This notion provides new perspectives for understanding the pathology of DIHS to correlate allergy with viral infection.

Perspectives in future

The recent technical approach of gene expression analysis has provided a great deal of information to reveal the mechanisms of drug hypersensitivity. Consequently, a new approach with *in silico* analysis is being developed for clarification of the exquisite interaction between drugs and HLAs or TCRs. The Ministry of Health, Labour and Welfare in Japan recently reported that there were 131 deaths over 2.5 years owing to drug hypersensitivity. To overcome this iatrogenic death, we should make an effort to clarify the mechanisms and to establish an effective treatment. We believe that these advances may avoid drug hypersensitivity reactions in the near future.

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Allergy

ORIGINAL ARTICLE

SKIN AND EYE DISEASES

Skin recruitment of monomyeloid precursors involves human herpesvirus-6 reactivation in drug allergy

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Keywords
$CD4^+$ T lymphocyte; drug-induced hypersensitivity syndrome; high-mobility group box-1; human herpesvirus-6, monocyte.

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Abstract

Background: In drug-induced hypersensitivity syndrome (DIHS), latent human herpesvirus (HHV)-6 is frequently reactivated in association with flaring of symptoms such as fever and hepatitis. We recently demonstrated an emergence of monomyeloid precursors expressing HHV-6 antigen in the circulation during this clinical course.

Methods: To clarify the mechanism of HHV-6 reactivation, we immunologically investigated peripheral blood mononuclear cells (PBMCs), skin-infiltrating cells, and lymphocytes expanded from skin lesions of patients with DIHS.

Results: The circulating monomyeloid precursors in the patients with DIHS were mostly $CD11b^+CD13^+CD14^-CD16^{high}$ and showed substantial expression of skin-associated molecules, such as CCR4. $CD13^+CD14^-$ cells were also found in the DIHS skin lesions, suggesting skin recruitment of this cell population. We detected high levels of high-mobility group box (HMGB)-1 in blood and skin lesions in the active phase of patients with DIHS and showed that recombinant HMGB-1 had functional chemoattractant activity for monocytes/monomyeloid precursors *in vitro*. HHV-6 infection of the skin-resident $CD4^+$ T cells was confirmed by the presence of its genome and antigen. This infection was likely to be mediated by monomyeloid precursors recruited to the skin, because normal $CD4^+$ T cells gained HHV-6 antigen after *in vitro* coculture with highly virus-loaded monomyeloid precursors from the patients.

Conclusions: Our results suggest that monomyeloid precursors harboring HHV-6 are navigated by HMGB-1 released from damaged skin and probably cause HHV-6 transmission to skin-infiltrating $CD4^+$ T cells, which is an indispensable event for HHV-6 replication. These findings implicate the skin as a cryptic and primary site for initiating HHV-6 reactivation.

Latent human herpesvirus (HHV)-6 is occasionally reactivated to manifest itself as virus-associated diseases that jeopardize the hosts. Drug-induced hypersensitivity syndrome (DIHS), also known as drug rash with eosinophilia and systemic symptoms, is a distinct entity of severe drug hypersensitivity because of its characteristic features (1, 2). HHV-6 reactivation has been found in more than 60% of such cases in association with unfavorable outcomes (3, 4), but the mechanisms remain unknown. To clarify this issue, we immunologically investigated peripheral and skin-infiltrating cells in DIHS. Our results suggest that skin recruitment of monomyeloid precursors navigated by high-mobility group box (HMGB)-1 is a crucial event in HHV-6 reactivation in DIHS.

Materials and methods

Patients

Twenty-seven patients with DIHS were enrolled in this study (Table 1). In addition, three normal male individuals and 24 patients with other types of drug eruptions (Stevens–Johnson syndrome (SJS), 9; toxic epidermal necrolysis (TEN), 3; maculopapular eruption (MPE), 8; and acute generalized

Table 1 DIHS patient profiles

Patient	Age/Gender	Culprit drug	Virus reactivation*
1	47/M	CBZ	HHV-6/CMV
2	49/M	CBZ	HHV-6
3	25/F	CBZ	HHV-6/HHV-7/CMV
4	73/F	CBZ	HHV-6/EBV/CMV
5	72/F	CBZ	HHV-6
6	51/M	CBZ	HHV-6/HHV-7
7	25/M	CBZ	HHV-7
8	89/F	CBZ	N.D.
9	42/F	CBZ/Phenytoin	HHV-6
10	68/M	Allopurinol	HHV-6
11	82/M	Allopurinol	HHV-7/EBV
12	54/M	Allopurinol	CMV
13	86/M	Allopurinol	HHV-6/CMV
14	53/F	Salazosulfapyridine	N.D.
15	46/F	Salazosulfapyridine	HHV-6
16	33/F	Salazosulfapyridine	HHV-6
17	48/F	Mexiletine	HHV-6/HHV-7
18	82/M	Mexiletine	HHV-7
19	57/F	Phenobarbital	HHV-6/CMV
20	60/M	Phenobarbital	HHV-6/CMV
21	43/F	Phenobarbital	HHV-6
22	42/M	Tribenoside	CMV
23	55/F	Tribenoside	HHV-6/CMV
24	18/F	Zonisamide	HHV-6/HHV-7
25	63/F	Zonisamide	HHV-6
26	40/M	Phenytoin	N.D.
27	69/F	Minocyclin	CMV

CBZ, carbamazepine, HHV, human herpesvirus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; N.D., no data.
*Antibodies against viruses that increased in titer in paired serum samples are indicated.

exanthematous pustulosis (AGEP), 4) were investigated. The study was performed according to the Declaration of Helsinki, and the study protocol was approved by the Hamamatsu University School of Medicine Ethical Committee. Written informed consent was obtained from all participants.

Reagents, monoclonal antibodies (mAbs), and culture medium

FITC-conjugated, PE-conjugated, and PerCP-conjugated mAbs against CD3, CD4, CD8, CD16, CD45, cutaneous lymphocyte antigen (CLA), HLA-DR, CD11b, CD13, CD14, CD34, CD117, and CD163 were purchased from BD PharMingen (San Diego, CA, USA). mAbs against CCR1 to CCR10 and CXCR1 to CXCR6 were obtained from R&D Systems (Minneapolis, MN, USA). A mouse anti-HHV-6 A and B variants mAb (clone 7C7 45/15) was obtained from Argene SA (Varilhes, France). A mouse anti-HMGB-1 mAb (clone J2E1) was obtained from ATGen (Gyeonggi-do, South Korea). Recombinant human HMGB-1 was obtained from R&D Systems Inc. Cells were cultured in RPMI 1640 medium (Life Technologies, Carlsbad, CA, USA) supplemented with L-glutamine, sodium pyruvate, 2-mercaptoethanol, nonessential amino acids (Life Technologies), and 10% heat-inactivated fetal calf serum or pooled human AB serum (cRPMI) as previously described (5). For expansion of T lymphocytes, the medium was supplemented with human recombinant IL-2 (R&D Systems) and/or anti-CD3/CD28 Ab-conjugated microbeads (T-cell Expander; Dynal, Copenhagen, Denmark) as previously reported (5).

Cell preparation

Blood samples were taken from the normal individuals and serially from the patients with DIHS during the disease course and after the resolution in some cases. Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized whole-blood samples by Ficoll–Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient centrifugation. After placement in plastic plates for 1–3 h, the adherent cells were used as a monocyte/monomyeloid precursor-rich fraction. For chemotaxis assay, the cells were recovered by gently scraping with a plastic cell scraper and transferred to a 15-ml conical tube to centrifuge 10 min at 1400 rpm and thus were transferred to new plates, maintained in culture with cRPMI containing GM-CSF at 10 ng/ml as a survival factor for prolonged explanted culture (6). CD4+ T cells were isolated from PBMCs with a CD4+ T-cell isolation kit (Miltenyi Biotec., Gladbach, Germany) according to the manufacturer's protocol. Skin-derived T cells were obtained by culturing skin samples for 72 h and expansion as described previously (5).

PCR for detection of HHVs

DNA was extracted from cultured cells using a DNeasy Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. For detection of HHVs including Epstein–Barr virus (EBV), HHV-6, HHV-7, and cytomegalovirus (CMV), appropriate DNA samples and positive DNA controls (HHV-6, HHV-7, and EBV) were amplified by multiplex nested PCR with specific primers as described previously (7). After amplification of specific virus gene fragments, the products were electrophoresed in a 4% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Flow cytometry

Aliquots containing 1×10^4 PBMCs were stained with fluorescence-conjugated mAbs against the target molecules and analyzed as described previously (8). HHV-6 antigen (Ag) detection was performed with an anti-HHV-6 Ab using a similar method to that described for intracytoplasmic cytokine staining (8).

Real-time horizontal chemotaxis assay

An optically accessible horizontal chemotaxis apparatus (EZ-TAXIScan; GE Healthcare UK Ltd., Buckinghamshire, UK) was used to evaluate the chemotactic activity of the cells, as previously described (9). The apparatus consisted of

front and back chambers containing cells and a chemoattractant, respectively, which were connected by a microchannel. Cells present within the microchannel (50 μm in length) during observation periods were successively recorded at 30-s intervals on a computer equipped with a video camera. Data were analyzed using the Image J software (National Institutes of Health, Bethesda, MD, USA) and the Manual Tracking plug-in produced by FP Cordelieres (Institut Curie; http://rsb.info.nih.gov/ij/plugins/manual-tracking.html).

Measurement of the plasma HMGB-1 concentration

The plasma HMGB-1 concentrations were measured using an HMGB-1 ELISA Kit (Shino-Test Co., Tokyo, Japan) according to the manufacturer's protocol.

Immunofluorescence staining

After fixation with acetone for 5 min, smear slides of cultured cells and snap-frozen skin sections (5 μm thick) were incubated with fluorochrome-conjugated Abs against the target molecules. Some specimens were incubated with a primary Ab and then incubated with a fluorescently tagged anti-mouse Ig secondary Ab after a blocking procedure. Nuclear counterstaining was performed with DAPI. The specimens were observed under a fluorescence microscope with filters for excitation at 490 and 540 nm. Specimens with omission of the primary Abs served as controls.

Electron microscopy

Samples were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed with OsO_4, and stained with uranyl acetate. After embedding in epoxy resin, ultrathin sections were examined under an electron microscope (JEM1220; Jeol Datum Ltd., Tokyo, Japan) and recorded with a CCD camera.

Statistical analysis

Pair-matched differences were analyzed by the Wilcoxon signed rank test, and comparisons among multiple samples were analyzed by Dunn's multiple comparison test and the Mann–Whitney U-test using the software GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Circulating HHV-6-positive monomyeloid precursors are recruited to the skin lesions of DIHS

We previously found a novel fraction (R1), which were distinct from a monocyte population (R2), in circulation during the course of DIHS (Fig. 1A) (10). The percentage of this fraction (R1) was increased in the early phase (<21 days after onset; mean ± SD, 9.9 ± 5.1%) and then returned to a normal level in the late phase (≥21 days after onset; 2.5 ± 1.1%) ($P = 0.0039$, Wilcoxon signed rank test). These cells were exclusively $CD13^+$ and $CD11b^+$ and largely $CD14^-$ (Fig. 1A, Fig. S1). They also variably, but substantially, expressed CD16, CCR4, CCR7, CXCR4, and CLA (Fig. 1A), with no or marginal expression of CCR1, 2, 3, 5, 6, 8, and 9 and CXCR1, 2, 3, and 5 (data not shown). A minor subset (~15%) of these cells also expressed CCR10. A macrophage marker, CD163, and stem cell markers, such as CD34 and CD117, were hardly detected (Fig. S1). These findings suggest that the cells belong to an immature/precursor type of the monomyeloid lineage in the bone marrow (11), and we designated them as monomyeloid precursors. The subset was marginally detected (<2%) in MPE ($n = 9$), SJS ($n = 8$), and TEN ($n = 3$). In these cells, we detected HHV-6 genome by multiplex nested PCR analysis (Fig. 1B, upper) and virus particles by electron microscopic analysis (Fig. 1C) as well as high expression of HHV-6 Ag by flow cytometric analysis (Fig. 1B, lower, Fig. S2).

Immunofluorescence staining revealed marked $CD13^+$ cells and $CD14^-$ or $CD11b^+$ in the dermis of the early stage of DIHS skin lesions ($n = 4$, Fig. 1D, left), where HHV-6 genome was simultaneously detected by PCR (Fig. 1B). However, lower infiltrates of these cells were observed in the dermis of AGEP skin lesions (Fig. 1D, Fig. S3), MPE, and SJS (data not shown), where HHV-6 genome was hardly detected (Fig. 1B).

HMGB-1 navigates monomyeloid precursors to the skin in DIHS

HMGB-1, a member of the damage-associated molecular pattern molecule (DAMP) family (12–16), may be a candidate for a chemoattractant for the monomyeloid precursors in DIHS. We measured the plasma concentrations of HMGB-1 at various time points in patients with DIHS ($n = 17$) as well as in patients with SJS ($n = 3$) and normal subjects ($n = 3$) (Fig. 2A). The plasma HMGB-1 levels in the early phase of DIHS were significantly higher ($n = 17$; mean ± SD, 8.61 ± 5.5 ng/ml) than those in the late phase ($n = 12$; 4.43 ± 1.45 ng/ml) and were much lower in the normal individuals (2.96 ± 0.53 ng/ml) (Dunn's multiple comparison test, $P < 0.05$ and $P < 0.01$, respectively). It was noted that two patients had extremely high levels of HMGB-1 in the early phase of DIHS (21.2 and 23.0 ng/ml), which were comparable to the levels in patients with sepsis (17). We also observed high levels of HMGB-1 in the patients with SJS (9.01, 6.05, and 6.0 ng/ml), consistent with a previous study (18).

To confirm that HMGB-1 is a functional navigator of the monomyeloid precursors, we performed real-time horizontal chemotaxis assays for the monocyte/monomyeloid precursor-rich fractions from the PBMCs of normal individuals ($n = 3$) and three patients with DIHS (#13, #15, and #16 in Table 1) using a cellular chemotaxis measurement device that offers advantages over existing methods by analyzing small numbers of cells. We observed significant chemotactic activity of recombinant HMGB-1 for healthy monocytes, following a Gaussian curve, with the maximum response at 8–40 ng/ml.

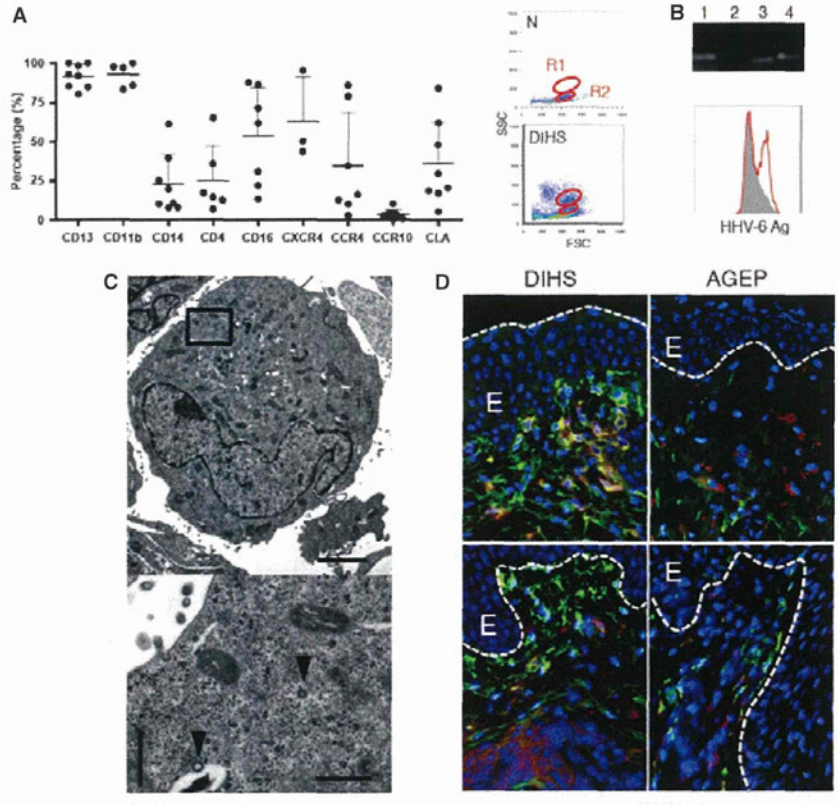

Figure 1 Characterization of circulating monomyeloid precursors. (A) Phenotype of the circulating monomyeloid precursors (R1) in DIHS patients ($n = 17$). These cells are distinct from monocytes (R2) in SSC levels. The percentages of the cells expressing the molecules investigated are indicated. Horizontal bars: mean values; vertical bars with small horizontal bars: standard deviations. N, normal. (B) Detection of HHV-6. Upper: PCR analysis of the HHV-6 genome in the monomyeloid precursor-rich fraction. Multiplex nested PCR for the detection of HHVs was performed. Lane 1, monomyeloid precursors; lane 2, normal PBMCs; lane 3, EBV-infected cells; lane 4, HHV-6 genome. Lower: flow cytometric analysis of HHV-6 Ag in the monomyeloid precursor-rich fraction as purified in the PCR analysis. AUTHOR: Please suggest whether the term 'patients' can be changed to 'patients' skin'.HHV-6 Ag expression was investigated in patients. Red line, monomyeloid precursors; gray shading: isotype. *X*-axis, HHV-6 Ag; *Y*-axis, cell number. (C) Electron microscopic observation of the monomyeloid precursors. The arrowheads indicate small round structures. Bars = 3 μm (upper) and 0.6 μm (lower). (D) Immunofluorescence staining of skin lesions in DIHS and AGEP. Double immunofluorescence staining was performed with FITC-anti-CD13 and PE-anti-CD14 Abs (lower panels) and with FITC-anti-CD13 and PE-anti-CD11b Abs (upper panels). Blue, DAPI; E, epidermis. Original magnification: ×400.

On the other hand, we found a significant chemotactic response of this molecule for the cells of the monomyeloid precursor-rich fractions only at 40 ng/ml. The effect was significantly abrogated by addition of a neutralizing Ab (Fig. 2B, right).

Next, we performed immunofluorescence staining of HMGB-1 in skin lesions. In normal skin, HMGB-1 was expressed in the nuclei of keratinocytes ($n = 2$, Fig. 2C, a–d), consistent with previous findings (19, 20). In contrast, strong expression of HMGB-1 in the cytoplasm of entire epidermal cells, infiltrating cells, and interstitial tissues of the dermis was found in DIHS lesions while diminished nuclear expression was noted ($n = 3$, Fig. 2C, e–h). These observations suggest that translocation of HMGB-1 has occurred. We observed that $CD13^+$ cells infiltrated adjacent to keratinocytes with strong HMGB-1 expression (Fig. 2C, h), suggesting chemoattraction for the monomyeloid precursors. On the other hand, HMGB-1 expression was observed in an upper layer of the epidermis in SJS skin lesions ($n = 3$, Fig. 2C, i–l), where $CD13^+$ cells showed marginal infiltration (Fig. 2C, l). The HMGB-1 expression area was larger in DIHS lesions than in SJS lesions and normal skin samples with significant differences (Fig. 2D), in accordance with greater relative HMGB-1 mRNA expression in DIHS skin ($n = 3$) than SJS skin ($n = 3$) although it was not statistically significant ($P = 0.1$) (Fig. S4).

HHV-6 is preferentially transmitted to $CD4^+$ T cells by monomyeloid precursors recruited to the skin

HHV-6 replicates exclusively in activated $CD4^+$ T cells and not in $CD8^+$ T cells or monomyeloid cells (21). Because no HHV-6 Ag is detected in circulating $CD4^+$ cells (10), we examined whether skin-resident T cells were infected with

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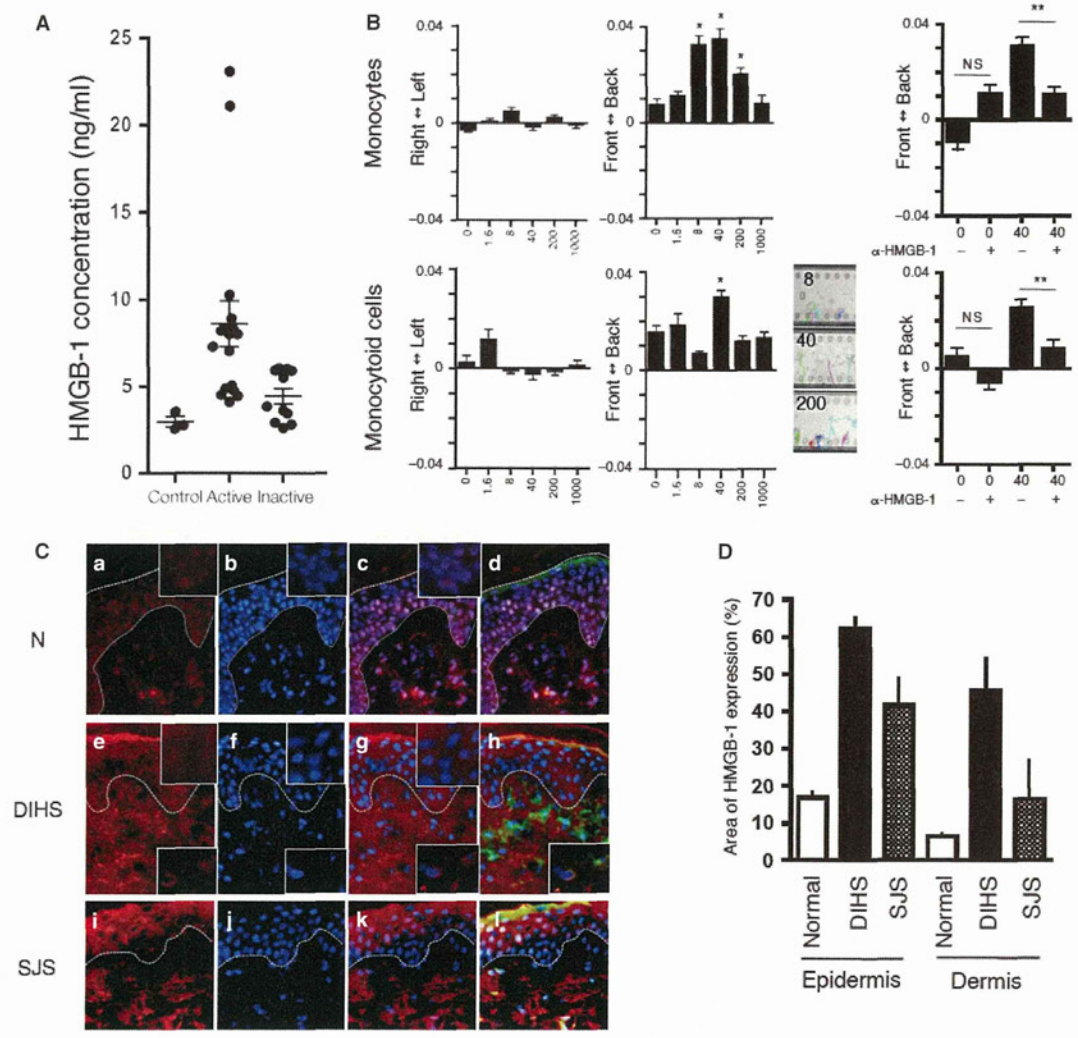

Figure 2 (A) Plasma concentrations of HMGB-1 in patients with DIHS Plasma samples were obtained from normal individuals (controls) and patients with DIHS in the early active (<day 21) and late inactive (≥day 21) phases and measured for their HMGB-1 concentrations (ng/ml) by ELISA. Horizontal bars: mean values; vertical bars with small horizontal bars: standard deviations. $*P < 0.01$, $**P < 0.05$, Dunn's multiple comparison test. (B) Chemotactic activity of HMGB-1 in the cells of the monocyte/monomyeloid precursor-rich fractions. The chemotactic response of the cells from healthy controls (upper) and patients with DIHS (lower) toward HMGB-1 at various concentrations was investigated with an EZ-TAXIScan device. The cell migration velocities in the right–left (left) and front–back (middle, right) directions were calculated and are depicted. The effects of an anti-HMGB-1 Ab on the cell migration are shown in the right panels. The data shown are means ± SD (≥500 cells/case; 3 cases/group are investigated.). The *X*-axis indicates the concentration of HMGB-1 (ng/ml), and the *Y*-axis indicates the velocity of the cell migration (μm/s). The picture panels show traces at around 3000 s in the presence of 8, 40, and 200 ng/ml of HMGB-1. $*P < 0.005$, $**P < 0.001$, Mann–Whitney U-test. (C) HMGB-1 expression in skin. Skin specimens from normal individuals (a–d, $n = 3$), patients with DIHS (e–h, $n = 3$) and patients with SJS (i–j, $n = 2$) were immunostained with an anti-HMGB-1 Ab (red, a, e, j) and anti-CD13 Ab (green, d, h, j). Nuclear staining was performed with DAPI (b, f, j). Representative data are shown. Merged images for HMGB-1/DAPI (c, g, h) and HMGB-1/DAPI/CD13 (d, h, l) are also shown. Original magnification, ×400; Insets, ×1000. Dotted line, epidermal–dermal junction. (D) HMGB-1 expression areas in skin. The data shown are means ± SD (20 areas/case; 3 cases/disease were investigated). The areas were calculated using Image J software. $*P < 0.05$, vs. normal and SJS skin, and $**P < 0.05$, vs. normal skin by the Mann–Whitney U-test.

HHV-6. We expanded skin-resident lymphocytes from three patients with DIHS (#3, #10, and #16 in Table 1) using a previously described method (5). The $CD4^+$ lymphocytes tended to be larger than the $CD8^+$ cells (Fig. 3A), similar to cytopathic lymphocytes after virus infection (22). In fact, HHV-6 Ag (Fig. 3C) and genome (Fig. 3B) were detected exclusively in $CD4^+$, but not in $CD8^+$, cells derived from DIHS skin lesions.

These findings prompted us to investigate whether the monomyeloid precursors harboring HHV-6 transmitted the

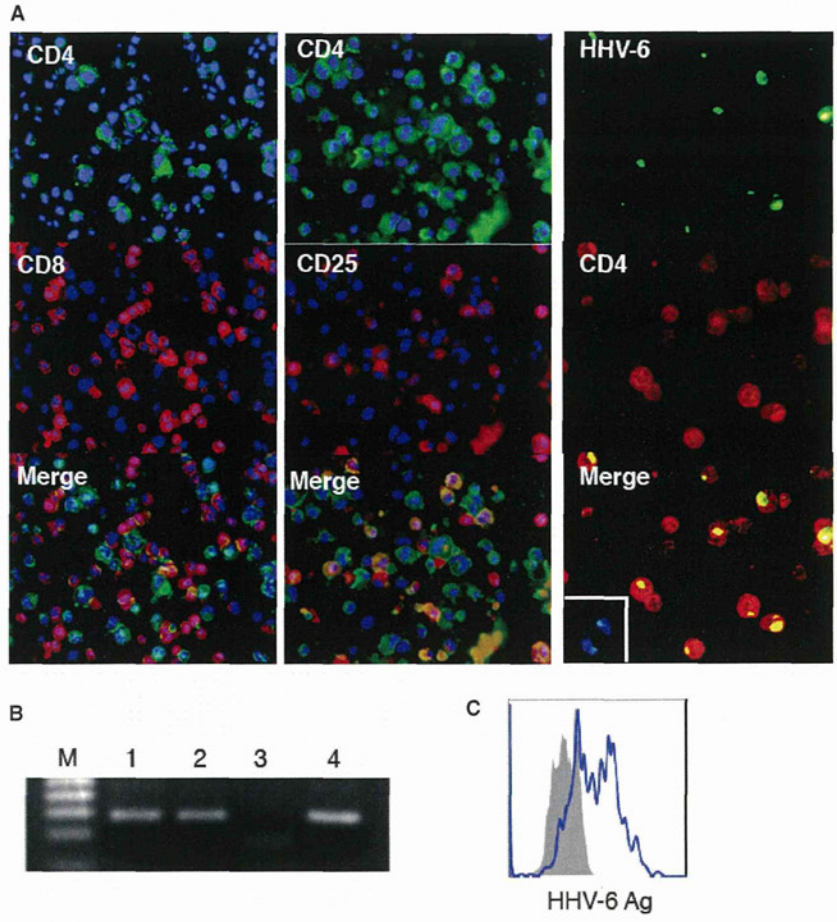

Figure 3 (A) Smear preparation of skin-resident T lymphocytes with immunofluorescence staining for CD4, CD8, CD25, and HHV-6 Ag. Blue, DAPI. Original magnification, ×400. (B) Multiplex nested PCR for detection of HHVs. Lane 1, DIHS skin lesion specimen; lane 2, DIHS skin lesion–resident CD4+ T cells; lane 3, SJS skin lesion–resident T lymphocytes; lane 4, HHV-6 genome as a positive control. (C) HHV-6 Ag expression (blue line) in skin-resident $CD4^+$ T cells. Gray shadow: isotype.

virus to skin-infiltrating $CD4^+$ T cells. We obtained monocytoid cell-rich fractions from cultures of PBMCs from two patients with DIHS (#16 and #19 in Table 1) at two different time points (days 14 and 21). $CD3^+$ T cells isolated from the normal individuals were mixed with each of the cultures containing the monocytoid cell-rich fractions, cultured for 5 days, and then analyzed for HHV-6 Ag by flow cytometry. HHV-6 Ag expression was higher in the 14-day monomyeloid precursor-rich fractions (74.5% and 63.7%) than the day-21 monomyeloid precursor-rich fractions (16.6% and 16.0%) from the two patients (Fig. 4A). After 5 days of coculture of the day-14 monomyeloid precursors and normal lymphocytes, 22% and 18% of the normal $CD4^+$ T cells became positive for HHV-6 Ag in two separate experiments. No HHV-6 Ag was detected in the normal $CD8^+$ T cells (Fig. 4A). On the other hand, HHV-6 Ag was insignificantly (~5%) detected in cocultures with the day-21 monomyeloid precursors. These findings indicate that the monomyeloid precursors preferentially potentiate the transmission of HHV-6 to a $CD4^+$ T-cell subset. Moreover, a high virus load in the monomyeloid precursors is likely to be a prerequisite for HHV-6 transmission.

We performed immunofluorescence staining to detect HHV-6 Ag in skin lesions biopsied at different time points (days 7 and 14 after onset) during the early phase in a patient with DIHS. In the skin lesion at day 7, the majority of cells infiltrating the skin were $CD3^+$ cells, while $CD13^+$ cells were sparsely observed (Fig. 4B, left upper). HHV-6 Ag was slightly detected (Fig. 4B, left lower). However, the skin lesion at day 14 revealed a higher number of infiltrating $CD13^+$ cells with coexpression of HHV-6 Ag as well as $CD3^+$ cells (Fig. 4B, right).

Discussion

Circulating monocytes in normal individuals are divided into two groups: a major $CD14^{high}CD16^-$ 'inflammatory' subset and a minor (<10% of total monocytes) $CD14^{low}CD16^+$ 'resident' subset. The cells in the latter subset differentiate into dendritic cells/macrophages to replenish the tissue-resident

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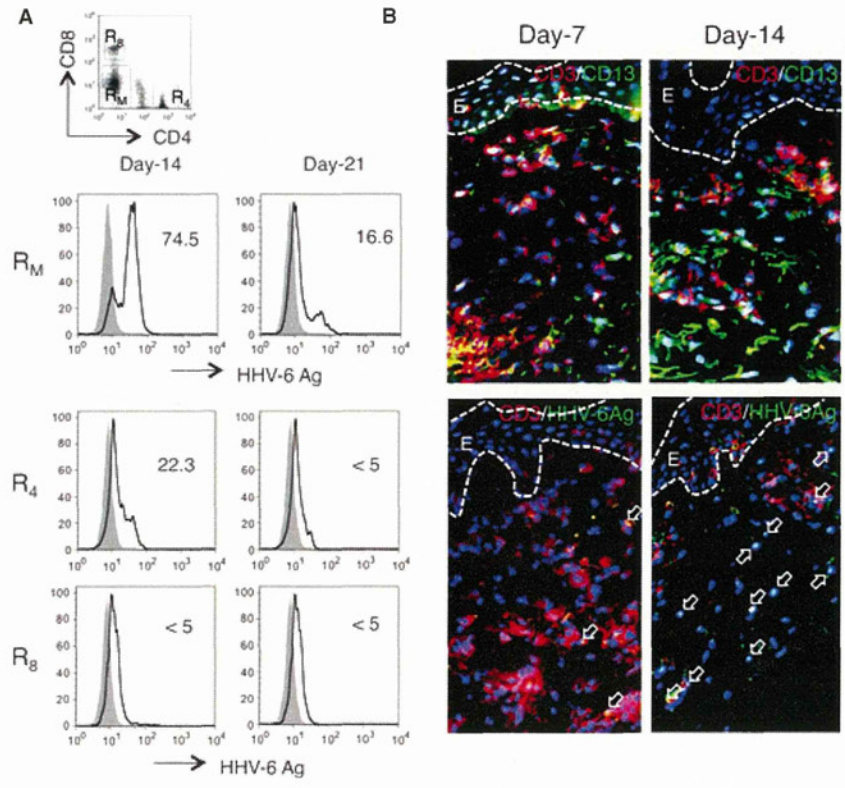

Figure 4 (A) *In vitro* HHV-6 transmission from monomyeloid precursors to allogeneic T lymphocytes. The monocytoid cell-rich fractions (Rm) from a patient with DIHS (#16 in Table 1) at days 14 and day 21 were cocultured with allogeneic T lymphocytes isolated from a healthy individual. After 5 days of culture, HHV-6 Ag expression was analyzed on the basis of the $CD4^+$ fraction (R4) and $CD8^+$ fraction (R8). The numbers indicate the percentages of HHV-6 Ag-positive cells in the total cells. The gray shadows indicate the isotype control. (B) $CD3^+/CD13^+$ cell infiltration and HHV-6 Ag expression in DIHS skin at days 7 and 14. Arrows indicate HHV-6 Ag expression. E, epidermis. Original magnification, ×400.

cells (23–25). On the contrary, the cells of the R1 population in DIHS comprised a minor $CD14^{high}CD16^-$ population and a major $CD14^-$ $CD16^+$ population that expressed skin-associated molecules such as CCR4, CLA markedly, and CCR10 partly, implying that they are precursors of a skin-resident subset. These cells were morphologically and phenotypically similar to monomyeloid precursors in the bone marrow (26), and some of them harbored HHV-6. Because some monomyeloid cells are latently infected with HHV-6 as virus reservoirs (27), the cells in the R1 population appear to have originated from monomyeloid precursors.

DAMPs released from damaged cells are cue signals for initiating immune responses in various organs through their activation after interacting with pattern recognition receptors and/or Toll-like receptors (12–16) and thus promote rapid recruitment of bone marrow-derived leukocytes to the target tissues for inflammation and regeneration in various aseptic inflammatory conditions (28–30), including SJS and TEN (31, 32). HMGB-1, one of the most well-known DAMP members, is a nonhistone protein with dual functions, namely transcriptional regulation by loose binding to chromatin inside cells, and a cue for inflammation outside cells with high potency to attract and activate various immunocompetent cells including monocytes and myeloid cells (12–16, 33). Our study first demonstrated high expression levels of HMGB-1 in blood and skin lesions in DIHS, with chemoattractant potency at 40 ng/ml of recombinant HMGB-1 *in vitro* for the cells of the monomyeloid percursor-rich fraction, suggesting its action as a navigator for skin recruitment of monomyeloid precursors in DIHS. Although a Gaussian curve of the chemotactic response to recombinant HMGB-1, as seen in healthy monocytes, was not found in monomyeloid precursors in DIHS, it might be due to diversity of the cell viability among these patients. Recently, other researchers found high expression levels of HMGB-1 in blood from patients with SJS (18). However, HMGB-1 was expressed at lower levels in SJS lesions than in DIHS lesions, suggesting different roles in each pathogenesis.

It has been demonstrated that a skin rash develops with simultaneous emergence of HHV-6 viremia in severely immunosuppressed patients (31). Therefore, we hypothesize that HHV-6 reactivation primarily starts in the skin of DIHS. Our observations constitute circumstantial evidence for this scenario. Immunohistochemical and immunological experiments proved close contacts of monomyeloid precursors and T cells in the skin and the presence of HHV-6 in skin-resident