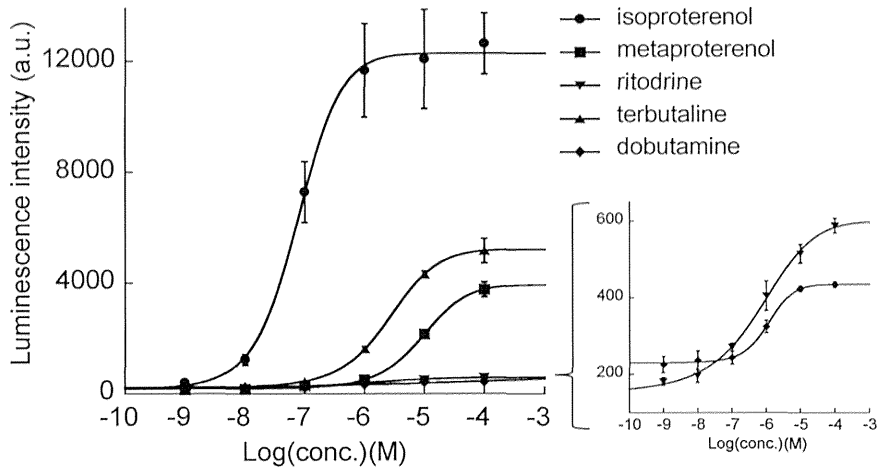

Fig. 3 In vitro plate assay upon stimulation with ligands using β2-adrenergic receptor, β-arrestin2 and FLuc as GPCR, β-arrestin, and split luciferase, respectively, which shows dose–response curves for the several agonists such as isoproterenol, metaproterenol, ritodrine, terbutaline, and dobutamine. Mean luminescence intensities were determined at each ligand concentration ($n = 3$). Error bars show standard deviations. Magnification of the region of low luminescence intensity is shown

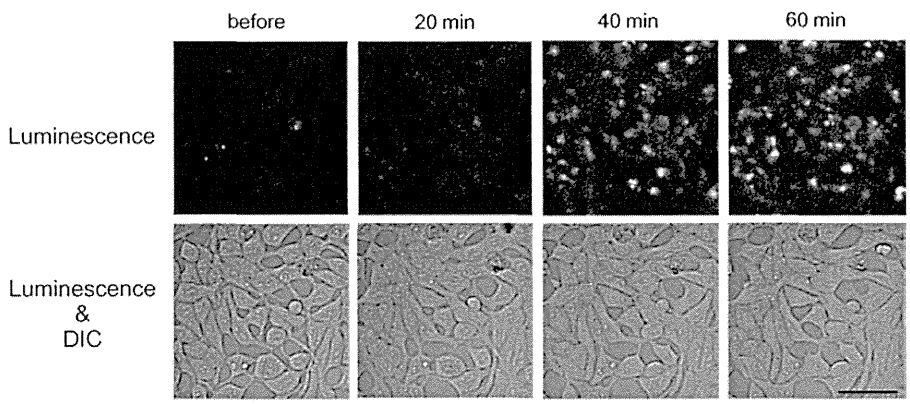

Fig. 4 Time-lapse images showing bioluminescence of HEK293 cells stably expressing somatostatin receptor2 and β-arrestin2 connected with split ELuc fragments. Cells were stimulated with 1×10^{-7} M of somatostatin. Injection time was defined at 0 min. Scale bar: 50 μm

3.2 Single-Cell Imaging with Bioluminescence Microscope

Live cell imaging enables the observation of the dynamics of protein–protein interaction over time (Fig. 4). Actually, we observed the internalization of GPCR with β-arrestin [16], which is useful for additional analysis of molecular mechanism:

1. Seed HEK293 cells of stable cell line on a PLL-coated 35-mm dish in DMEM ph(+)P/S(+) at 37 °C in an atmosphere of 5 % CO_2, and grow up to 50–70 % confluence (*see* **Note 22**).
2. Replace the medium with HBSS or DMEM ph(−)P/S(+) containing 4–10 mM of D-luciferin. Incubate the dish at 37 °C for 15 min (*see* **Note 23**).

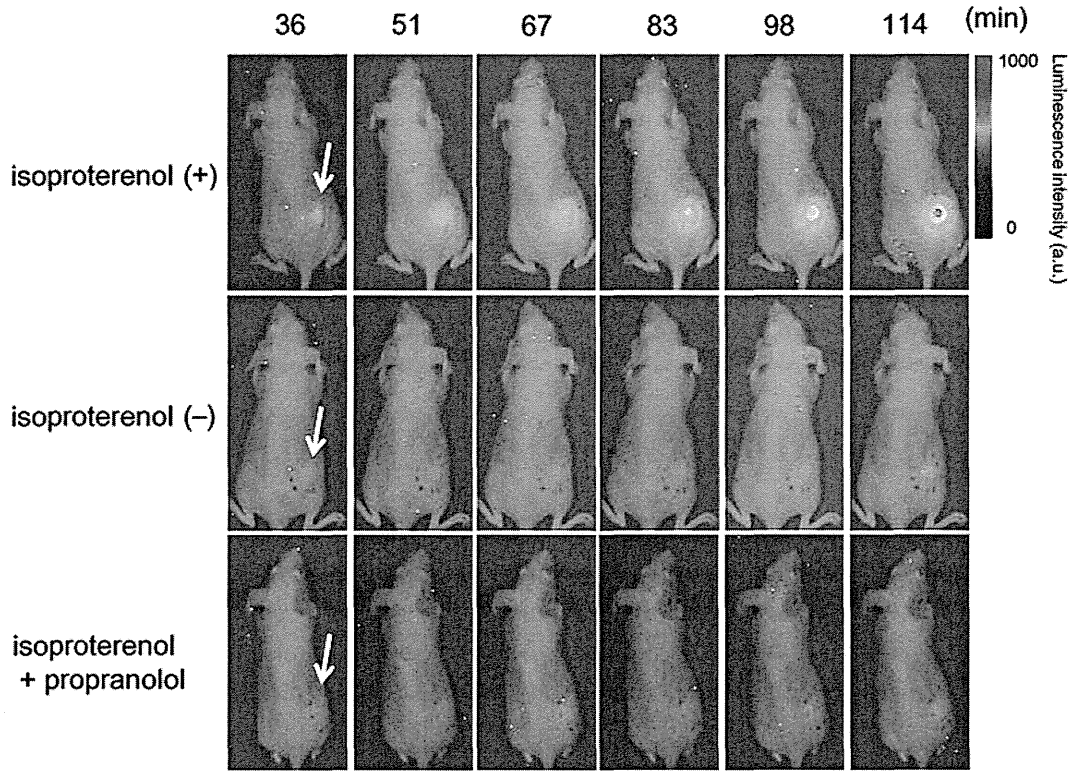

Fig. 5 In vivo imaging of G protein activity with cell implantation. Mice were implanted subcutaneously with HEK293 cells stably expressing β2-adrenergic receptor and β-arrestin2 fused to split FLuc fragments with D-luciferin. Images were taken at the indicated times after i.p. injection of isoproterenol. Arrows indicate the locations of cell implantation

3. Put the dish on the stage set at 37 °C and adjust the focus.
4. Take the bioluminescence and bright field images of cells on time lapse with MetaMorph software (*see* **Notes 24** and **25**).
5. After taking a few images, add the agonist to the dish (*see* **Note 26**). Continue the measurement.

3.3 In Vivo Imaging with Cell Implantation

Here, we describe the method of in vivo imaging with cell implantation. We implanted cells with D-luciferin on the back of mice because we avoided the dispersion or metabolism of D-luciferin during measurement for a few hours [16]. Using this method, we can evaluate the distribution of therapeutic reagents (Fig. 5):

1. Seed HEK293 cells of stable cell line on a 10-cm dish in DMEM ph(+)P/S(+) at 37 °C in an atmosphere of 5 % CO_2, and grow up about 90 % confluence.
2. After washing with PBS, harvest the cells to the microtube using a rubber scraper.

3. After centrifugation, remove the supernatant. Then suspend the cells in 150 μL of PBS containing D-luciferin (90 mg/kg of body weight) (*see* **Note 27**). Put cells on ice immediately before use.
4. After anesthesia of a mouse with isoflurane, implant the cells subcutaneously on the back of the mouse.
5. Inject 150 μL of the agonist in PBS intraperitoneally (i.p.).
6. After 30 min, put the mouse into a black box of in vivo imaging system under anesthesia. Then take the images using an EM-CCD camera (*see* **Note 28**).

3.4 In Vivo Imaging with HTV

HTV, a method to introduce the naked plasmid DNA into the liver and other organs, such as the kidney, spleen, lung, and heart, of living animals [18–20], is convenient and safe compared to the infection of virus and creation of transgenic mice because one needs only plasmid DNA for the preparation. In principle, HTV is characterized by the large amount of injection solution and the injection rate with high speed. These cause increased pressure in the inferior vena cava and then the hepatic vein, resulting in the formation of pores in the hepatocyte membrane. Consequently, plasmid DNA can enter the cells. In HTV method, unlike the cell implantation explained above, it is necessary to administer D-luciferin i.p. because the substrate must be delivered into the organ. In this case, D-luciferin is metabolized rapidly and consumed after i.p. injection. Therefore, the signal shows efficacy of the agonist injected in the endogenous system just at the time of i.p. injection of D-luciferin (Fig. 6). The two methods should be discriminated for the study:

1. Dissolve 20 μg each of two plasmids encoding GPCR and β-arrestin connected with split luciferase probes in 1.5–2 mL of saline (about one-tenth of the body weight of mice).
2. After anesthesia of a mouse with isoflurane, inject the mixture of plasmids at once through the tail vein of the mouse within 5–10 s at a constant rate (*see* **Notes 29–31**).
3. After 18–24 h, inject 150 μL of agonist and D-luciferin (600 mg/kg of body weight) in PBS i.p.
4. Put the mouse into a black box of in vivo imaging system under anesthesia. Take the images using an EM-CCD camera.

4 Notes

1. The cDNAs of GPCR and β-arrestin connected with split luciferase probes are usually inserted into expression vectors of pcDNA4/V5-His and pcDNA3.1/myc-His, respectively, for the establishment of a stable cell line.

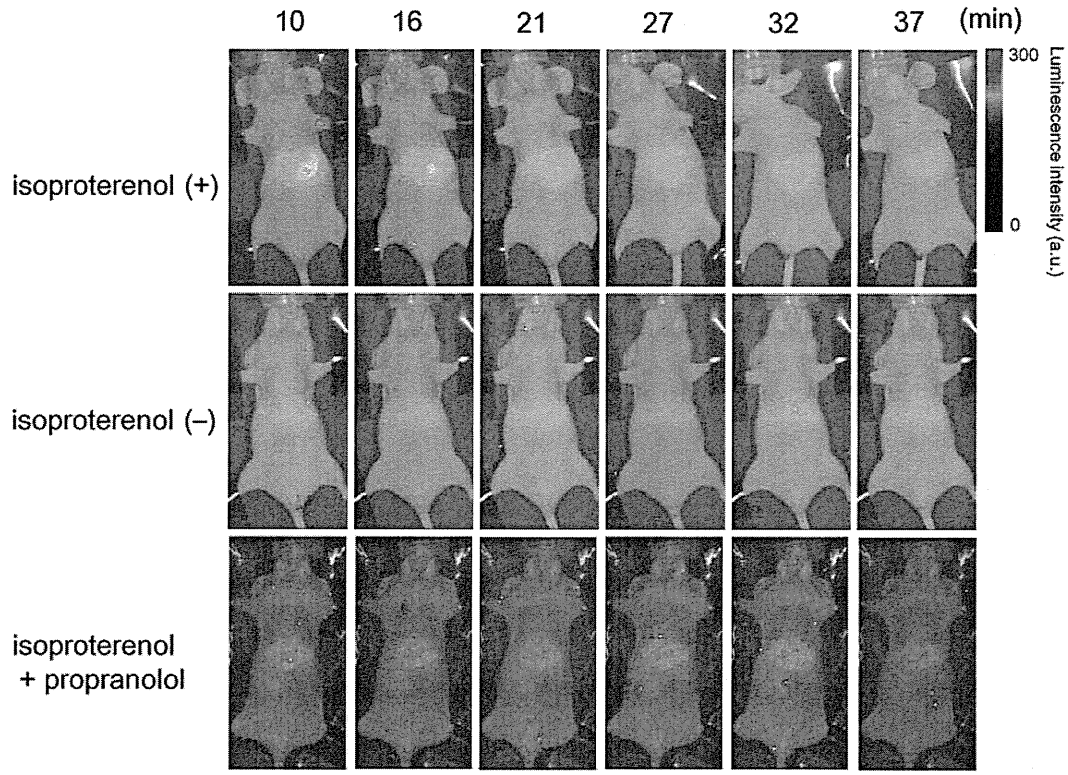

Fig. 6 In vivo imaging of G protein activity with HTV method. The mice were treated with HTV using cDNA plasmid of β2-adrenergic receptor and β-arrestin2 fused to split FLuc fragments. D-Luciferin and the ligand were injected i.p. Images were taken at the indicated times after the injection

2. The intensity of split ELuc probe on the stimulation tends to be higher than that of FLuc. However, for in vivo imaging, FLuc should be used because emission light with longer wavelength offers good tissue penetration as a result of the minimal interference from hemoglobin [21, 22].
3. Click beetle in red (Caribbean *Pyrophorus plagiophthalamus*, CBR, $\lambda_{max} = 613$ nm) is available as a red-emitting luciferase for in vivo imaging. However, the activation ratio of split CBR probe was much worse [16].
4. Another tool to make the emitting wavelength longer for in vivo imaging is mutation of luciferase. We tried two red-shifted mutants of FLuc (S284T and A348V) for split luciferase. However, the intensity decreased drastically [16].
5. To date, we have demonstrated that many GPCRs are useful for the complementary strategy, such as somatostatin type 2 receptor, apelin receptor, adrenergic receptors (α2a, β2), cholecystokinin B receptor, endothelin receptors (types A, B), μ-opioid receptor, endothelial differentiation G protein-coupled receptor 3, and angiotensin II receptor type I [14–17].

6. The cDNAs of GPCRs and β-arrestins were cloned from the human placenta cDNA library (Toyobo Co. Ltd.) and a human brain cDNA library (Takara Bio Inc.).
7. In the case of the interchanging pair (GPCR with N-terminal fragment and β-arrestin with C-terminal fragment), the activation ratio upon stimulation by ligands got worse [16].
8. The dissection site of ELuc was determined by semirational library screening [14].
9. Lipofectamine 2000 is more toxic than TransIT-LT1 in our experience.
10. The concentration of heat-inactivated FBS was between 1 and 10 %.
11. We used TriStar LB941 (Berthold Technologies GmbH and Co.). Other plate readers for luminescence are useful.
12. The entire microscope is covered completely with a black box. The room light is turned off during measurement for avoidance of leak of light.
13. Sensitivity of EM-CCD camera is important to obtain clear bioluminescence images.
14. Localizations of GPCR and β-arrestin can be checked using immunofluorescence [14–16].
15. We usually used 0.8–2 mg/mL G418 and 0.04–0.5 mg/mL Zeocin for selection and 0.8 mg/mL G418 and 0.04 mg/mL Zeocin for culture.
16. Z' factor is defined as evaluation of the quality of the HTS assays [23]. The equation is the following:

$$Z' \text{ factor} = 1 - \frac{\left(3 \times SD_{100\%} + 3 \times SD_{0\%}\right)}{\left|Av_{100\%} - Av_{0\%}\right|}$$

Therein, $Av_{100\%}$ and $Av_{0\%}$, respectively, represent the average values of positive and negative controls, and $SD_{100\%}$ and $SD_{0\%}$, respectively, denote the standard deviations of positive and negative controls. More than 0.5 of Z' factor is necessary for valid screening [23]. Most of our systems are satisfied with this condition.

17. Before preparation, the experimental plan should be designed well because it is related to the number of wells needed and so on. For example, we usually performed all measurements 3–6 times with different wells of plates in quantitative assay for calculating the standard deviations. In addition, the range of concentrations of ligand was decided to show the sigmoidal dose–response curve. When HTS is conducted using large amount of chemicals, the number of measurements can be reduced to save time.

18. We split all of the approximately 90 % confluence of HEK293 cells expressing split luciferase probes on a 10-cm dish equally to each well of a 96-well plate.
19. We strongly recommend the use of an 8-channel or 12-channel pipette to add the solution to the wells of the plate for accurate volume and saving time.
20. When the inhibition assay is conducted, the antagonist is added to the wells before the addition of the agonist.
21. The exposure time depends on the instruments used. We set the time at 2 s per well. Sufficient signals were obtained.
22. HEK293 cells are easily detached on the surface of the dish. Care is advised when changing the medium. PLL can help in the attachment of the cells. PLL-coated dishes are commercially available. They can be prepared by hand. The protocol is the following: 1 mL of PLL was added to a 35-mm dish; the dish was incubated at room temperature for 30 min. Then the dish was washed three times with PBS. The PLL-coated dishes should be prepared immediately before use.
23. Alternatively, the dish can be incubated on the stage incubator of the microscope.
24. The exposure time depends on the sensitivity of the used EM-CCD and the intensity of split luciferase probe. We usually set the time at 3–5 min.
25. The focuses of bioluminescence and bright field differ in our microscopic system. Therefore, we took several images of different Z positions in a bright field.
26. Care must be taken not to touch the dish when adding the solution of agonist because it must be done in a dark place. In a black box, we used a handy light and a microsyringe, not a pipette for addition.
27. The cell number was about 5×10^6 cells.
28. The time points of measurement depend on GPCR and agonists. In the case of β2-adrenergic receptor and isoproterenol, the signal of luminescence started to increase 30 min after the administration of the agonist.
29. Intravenous (i.v.) injection should be done in a way that you think is best. We used a mouse restraint made by hand from a centrifuge tube. Of course, restraints are commercially available. Furthermore, it is important to enlarge the blood vessel of the tail vein, such as by wiping the vein with 70 % ethanol, clipping the root of the vein, warming up with light, and so on. We used a needle of 31 G size. We recommend a trial experiment with full-length luciferase before the use of split luciferase probe. At this time, 10 μg of plasmid was sufficient to visualize the signal in our system.

30. It might fail if the pressure during the injection goes up. Constant resistance was continued at the successful injection.
31. Immediately after i.v. injection, something wrong might occur with the mouse, such as slow movement or convulsion. At that time, it was helpful to warm the mouse with a hand or a heater and do heart massage for the recovery of the mouse. If mice often die after i.v. injection, then the usage of a specific solution for the injection, such as TransIT Hydrodynamic Delivery System (Mirus Bio Corp.), might be helpful.

Acknowledgment

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SHORT COMMUNICATION

Stevens-Johnson Syndrome Associated with Mogamulizumab-induced Deficiency of Regulatory T cells in an Adult T-cell Leukaemia Patient

Tetsuya Honda[1,5], Masakatsu Hishizawa[2], Tatsuki R. Kataoka[3], Katsuyuki Ohmori[4], Akifumi Takaori-Kondo[2], Yoshiki Miyachi[1] and Kenji Kabashima[1]*

*Departments of [1]Dermatology, [2]Hematology and Oncology, [3]Diagnostic Pathology, [5]Innovation Center for Immunoregulation Technologies and Drugs, Graduate School of Medicine, Kyoto University, Shogoin Kawara-cho 54, Kyoto 606-8507, and [4]Department of Laboratory Medicine, Kyoto University Hospital, Kyoto, Japan. *E-mail: kaba@kuhp.kyoto-u.ac.jp*

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Stevens-Johnson syndrome (SJS) is a rare but extremely severe drug-induced eruption characterised by widespread necrosis or apoptosis of the epidermis (1). The main causal factor for SJS is an aberrant activation of CD8 T cells. Although the dysfunction of the regulatory T cells (Treg) is considered a possible cause of excess CD8 T cells activation (2), the actual significance of Tregs *in vivo* remains unknown.

CASE REPORTS

A 54-year-old man with relapsed adult T-cell leukaemia-lymphoma (ATL) (stage IVB) was referred to our clinic with multiple erythema and small red papules on his lower legs (Fig. 1a). Eight days earlier (day 0) he had started weekly treatment with mogamulizumab, a humanised anti-CC chemokine receptor 4 (CCR4) monoclonal antibody that depletes $CCR4^+$ tumour cells in ATL patients. Histological examination of the papules exhibited inflammatory cell infiltration in the perivascular and interface between the dermis and the epidermis (Fig. 2a). $CD8^+$ cells were mainly located in the perivascular region, and forkhead box P3 $(FOXP3)^+$ Tregs existed in the interface (Fig. 2b, c). Necrosis of epidermal cells was not detected. We diagnosed the skin rash as a drug-induced eruption by mogamulizumab. As topical difluprednate treatment did not control the skin rash, we discontinued the third course of mogamulizumab treatment, and started 30 mg of oral prednisolone treatment on day 12.

Although the erythema and papules temporarily improved after the systemic steroid therapy, the clinical manifestations spread over his trunk and extremities (Fig. 1b) on day 28 accompanied by severe conjunctivitis, erosion and swelling of oral mucosa (Fig. 1c), and high fever. Histological examination on day 28 revealed severe epidermal cell necrosis and vacuolar changes with significant numbers of $CD8^+$ cells in the interface in accord with the absence of Tregs (Fig. 2d–f). Flow cytometry analysis revealed that Tregs in blood had disappeared by day 17 (Fig. 3a, b). We diagnosed the patient as SJS induced by mogamulizumab. We initiated pulse therapy with methylprednisolone (500 mg/day × 3 days) plus tacrolimus (1.5 g/day × 2 days), followed by methylprednisolone treatment (70 mg/day). After these treatments, the skin rash gradually improved. We carefully tapered the dose of methylprednisolone to 30 mg/day. Even after the discontinuation of mogamulizumab

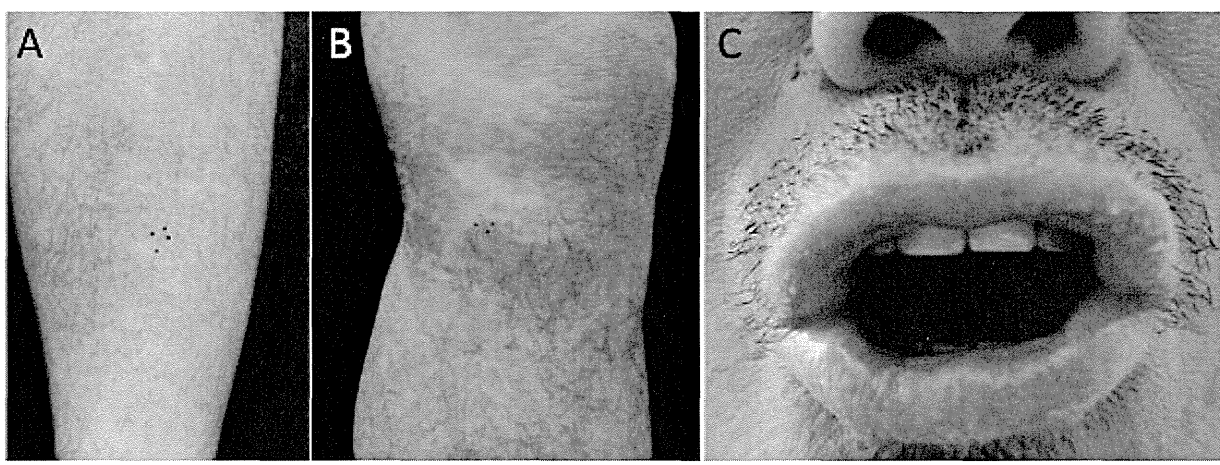

Fig. 1. Multiple erythema and small papules on day 8 (a). Erythema multiforme-like erythema on extremities (b), and erosion and swelling of oral mucosa on day 28 (c).

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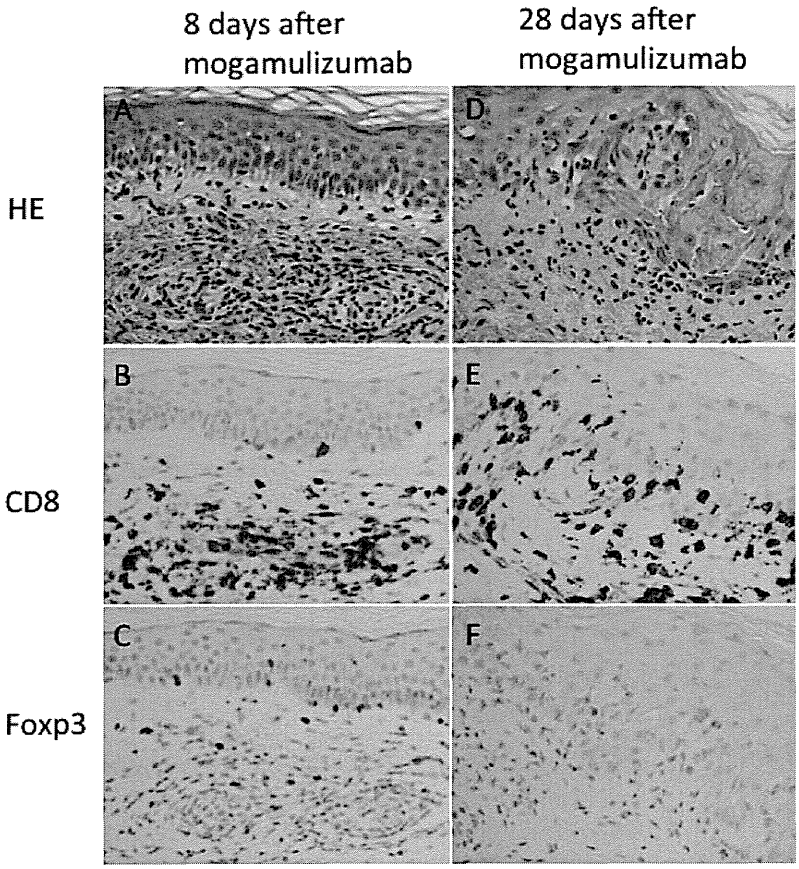

Fig. 2. Histological and immunohistochemical (IHC) findings 8 (a–c) and 28 days (d–f) after mogamulizumab treatment. (a, d) Haematoxylin and eosin staining. IHC for CD8 (b, e) and FOXP3 (c, f). Original magnification: × 40.

therapy, the patient achieved a complete remission in ATL. He was discharged on day 81.

DISCUSSION

Mogamulizumab was approved in Japan in March 2012 as a novel therapy for relapsed or refractory ATL, because tumour T cells of ATL patients are CCR4$^+$ in nearly 90% of cases (3). Although it significantly improves the clinical symptoms in ATL patients, it sometimes induces severe adverse effects of the skin, such as SJS and toxic epidermal necrolysis (4). In the current case, the development of SJS was inversely correlated to the presence of Tregs in the skin. Tregs were probably depleted by mogamulizumab, because CCR4 is highly expressed on Tregs as well as on ATL tumour cells (4, 5). Although more cases are to be analysed, our case may provide *in vivo* evidence that absence of Treg functions in skin is the primary cause of SJS, at least during the treatment with mogamulizumab.

It has been reported that the skin lesion by mogamulizumab usually developed after the fourth or subsequent infusion (4, 6), while the skin lesion in our case developed just after the second infusion. Thus far, it remains unknown what kind of factors determine the onset or severity of adverse skin reaction by mogamulizumab (6). Further research is required to reveal the mechanism.

The authors declare no conflict of interest.

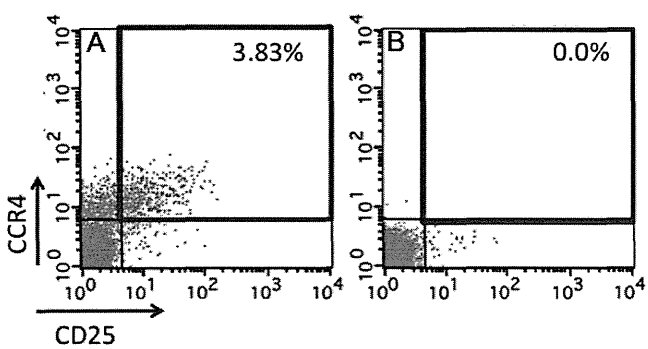

Fig. 3. Flow cytometry analysis for CCR4$^+$CD25$^+$CD4$^+$CD3$^+$ non-tumour cells in peripheral blood before (a) and 17 days (b) after the mogamulizumab therapy. Data are gated on CD4$^+$CD3$^+$ cells.

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SHORT COMMUNICATION

DIHS/DRESS with Remarkable Eosinophilic Pneumonia Caused by Zonisamide

Rintaro Shibuya[1], Hideaki Tanizaki[1], Saeko Nakajima[1], Itsuko Koyanagi[2], Tatsuki R. Kataoka[2], Yoshiki Miyachi[1] and Kenji Kabashima[1]*

*[1]Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Shogoin, Kawahara-cho, Sakyo-ku, Kyoto 606-8507, and [2]Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan. *E-mail: kaba@kuhp.kyoto-u.ac.jp*

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Drug-induced hypersensitivity syndrome (DIHS), also known as drug reaction with eosinophilia and systemic symptoms (DRESS), is a rare and severe skin disease associated with systemic findings, such as fever, eosinophilia, lymphadenopathy, and internal organ involvement that typically develops 2 to 6 weeks after the drug intake, following a prolonged course with frequent flare-ups and relapses over weeks or even months after discontinuing the drug. This syndrome has been reported to be associated with the intake of anticonvulsants, sulfonamides and allopurinol (1). Several mechanisms are involved in its pathophysiology, such as drug toxicity, immunological imbalance and reactivation of the herpes virus family members. Recently, a certain type of human leucocyte antigen (HLA) was identified as a predisposing factor in DIHS/DRESS. Here, we describe a case of DIHS/DRESS with remarkable eosinophilic pneumonia caused by zonisamide.

CASE REPORT

A 46-year-old woman was referred to our hospital with a spiking fever, dry cough and skin rash. She had a past medical history of subarachnoid haemorrhage due to hereditary haemorrhagic telangiectasia (HHT) and had commenced zonisamide 8 months before. She had been administered with zonisamide for 2 months, and followed by a washout period of 4 months. On the 41st day of the second course of treatment, she developed fever with chills, dry cough, and progressive non-pruritic maculopapular eruption with mucosal involvement (Fig. 1 a, b).

She was hospitalised for further evaluation. Blood tests revealed white blood cell count 8,800/mm^3 (11% eosinophils, 49% neutrophils and 27% lymphocytes) and platelet count 227,000/mm^3. Liver function test results were elevated: asparate aminotransferase (AST) 67 U/l, alanine aminotransferase (ALT) 133 U/l; alkaline phosphatase (ALP) 220 U/l. Serologic tests for viral infections, including hepatitis B, hepatitis C, Epstein-Barr virus (EBV), cytomegalovirus, and human herpes virus 6 (HHV6) were negative. A chest radiograph did not reveal a pulmonary infiltration. A skin biopsy of the erythematous eruption on her abdomen exhibited spongiosis and vacuolar degeneration of epidermal basal keratinocytes. Lymphocytic perivascular infiltration was present in the dermis (Fig. 1 c, d). Immunohistochemical staining showed that lymphocytes were CD8$^+$ lymphocytes with sparse Foxp3 positive lymphocytes in the upper dermis (Fig. 1 e, f).

Based upon the patient's clinical, laboratory, and pathological findings, DIHS/DRESS was suspected. Zonisamide was discontinued immediately and topical steroids were initiated. On the 11th day of hospitalisation, however, her dry cough was exacerbated. Laboratory tests revealed leucocytosis (11,800/μl) with eosinophilia (3,800/μl) and atypical lymphocytosis. HHV6 IgG titre was increased from × 20 (at day 4) to × 120 (at day 17), confirming a reactivation of HHV6. Drug-induced lymphocyte stimulation test (DLST) for zonisamide was positive (a stimulation index of 338.2 %). Chest computed tomography (CT) showed multiple bilateral nodular lesions with surrounding ground-glass-opacity halo (Fig. 1g). Eosinophilic pneumonia (EP) due to zonisamide was suspected and systemic steroid therapy (0.5 mg/kg/day of prednisolone) was commenced. Peripheral eosinophils decreased and her pulmonary lesions improved after one week. Oral prednisolone was tapered and she was discharged on the 35th day of hospitalisation.

DISCUSSION

The diagnosis of DIHS/DRESS is based upon clinical and laboratory findings. The European Registry of Severe Cutaneous Adverse Reaction (RegiSCAR) study group has devised a scoring system (2). Our case was scored as "7" which is classified as a "definite" case (Table SI[1]). Although, bronchoscopy was not successfully performed due to cardiopulmonary arrest during the procedure, the findings of high eosinophil counts and pneumonia developing 2 weeks after the onset of DRESS, along with the dramatic clinical response to glucocorticoids, strongly suggest that EP was a part of DRESS/DIHS rather than a separate adverse effect of zonisamide. Although pulmonary involvement is rarely reported, it may lead to life threatening adult respiratory distress syndrome (3, 4).

The pathogenesis of DIHS/DRESS remains unclarified, but reactivation of the herpes virus family has been reported at the onset of DIHS/DRESS (5). Tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) secreted by the anti-EBV CD8$^+$ T lymphocytes in cutaneous and visceral lesions may contribute to the development of DIHS/DRESS at the early stage (6). One study has demonstrated a switch in the predominant drug-specific proliferating T-cell population in the course of DIHS/DRESS; CD8$^+$ lymphocytes were predominant initially, whereas CD4$^+$ lymphocytes and regulatory T (Treg) cells (CD4$^+$CD25$^+$Foxp3$^+$) proliferated at the recovery stage (7). In our case, immunohistochemical staining showed abundant CD8$^+$ lymphocytes and few Treg cells infiltration in the upper dermis, reflecting the acute phase of DIHS/DRESS.

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1863

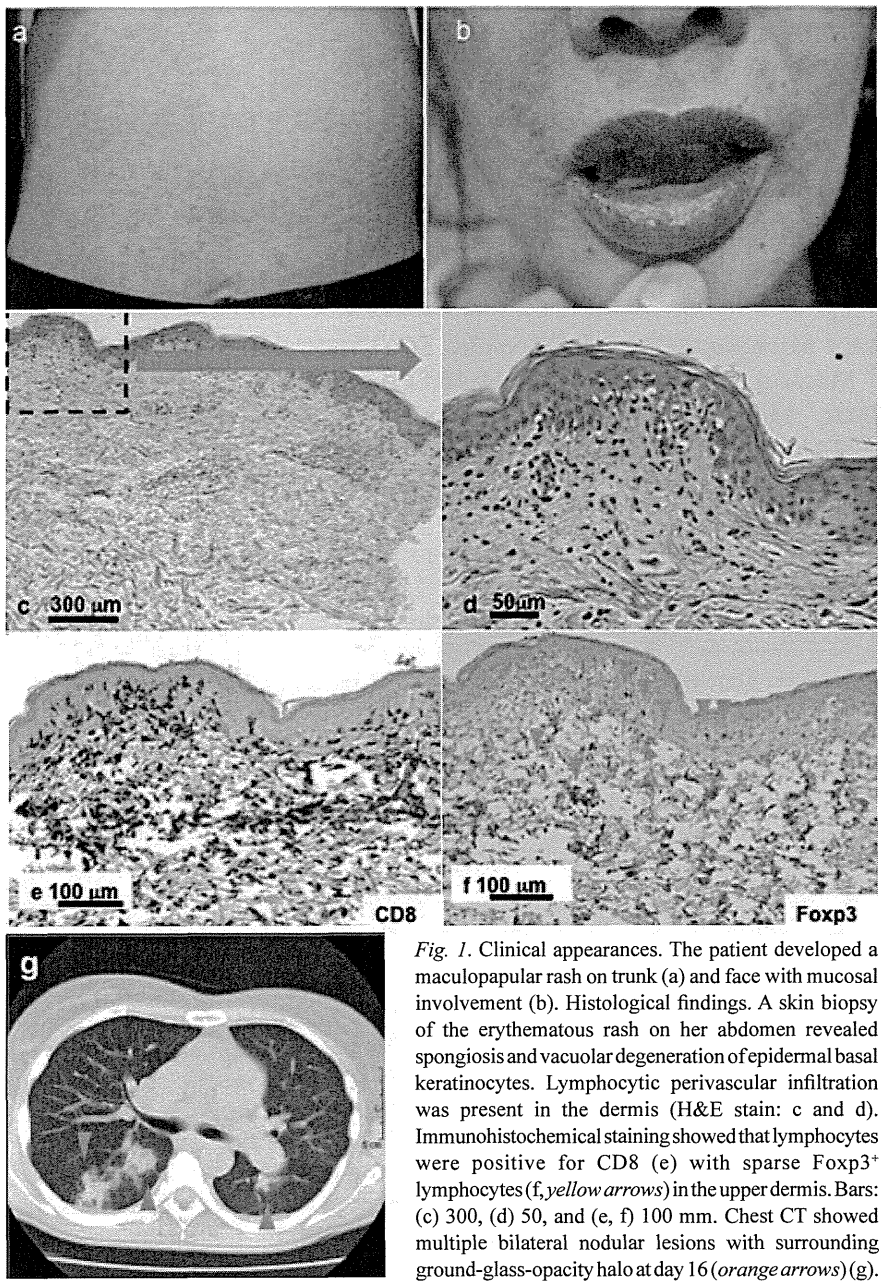

Fig. 1. Clinical appearances. The patient developed a maculopapular rash on trunk (a) and face with mucosal involvement (b). Histological findings. A skin biopsy of the erythematous rash on her abdomen revealed spongiosis and vacuolar degeneration of epidermal basal keratinocytes. Lymphocytic perivascular infiltration was present in the dermis (H&E stain: c and d). Immunohistochemical staining showed that lymphocytes were positive for CD8 (e) with sparse $Foxp3^+$ lymphocytes (f, *yellow arrows*) in the upper dermis. Bars: (c) 300, (d) 50, and (e, f) 100 mm. Chest CT showed multiple bilateral nodular lesions with surrounding ground-glass-opacity halo at day 16 (*orange arrows*) (g).

At present, we do not have clear evidence how EP was induced by zonisamide in DRESS/DIHS. Pulmonary involvement in DIHS/DRESS is known to be induced by a certain drug (3, 4). We consider that pulmonary involvement in DIHS/DRESS is not related to specific drugs only but may be related to the patient's underlying condition, such as HHT. Antigen-presenting cells (e.g. alveolar macrophages) are known to ingest drugs and present them to T helper cells to release interleukin-5, resulting in eosinophil proliferation (8). Therefore, in patients with HHT, blood vessels tend to be fragile and prone to bleeding (9), which may cause the accumulation of zonisamide into the lung and stimulate macrophages to initiate the immunologic cascade. Clinicians should be aware of the possible involvement of the lung in the course of DIHS/DRESS.

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The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Sequelae in 145 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms: Survey conducted by the Asian Research Committee on Severe Cutaneous Adverse Reactions (ASCAR)

Yoko KANO,[1] Mikiko TOHYAMA,[2] Michiko AIHARA,[3] Setsuko MATSUKURA,[4] Hideaki WATANABE,[5] Hirohiko SUEKI,[5] Masafumi IIJIMA,[5] Eishin MORITA,[6] Hiroyuki NIIHARA,[6] Hideo ASADA,[7] Kenji KABASHIMA,[8] Hiroaki AZUKIZAWA,[9] Hideo HASHIZUME,[10] Keisuke NAGAO,[11] Hayato TAKAHASHI,[11] Riichiro ABE,[12] Chie SOTOZONO,[13] Michiko KUROSAWA,[14] Yumi AOYAMA,[15] Chia-Yu CHU,[16] Wen-Hung CHUNG,[17] Tetsuo SHIOHARA[1]

[1]Department of Dermatology, Kyorin University School of Medicine, Tokyo, [2]Department of Dermatology, Ehime University Graduate School of Medicine, Ehime, [3]Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, [4]Department of Dermatology, Yokohama City University Medical Center, Yokohama, [5]Department of Dermatology, Showa University School of Medicine, Tokyo, [6]Department of Dermatology, Shimane University School of Medicine, Izumo, [7]Department of Dermatology, Nara Medical University School of Medicine, Kashihara, [8]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, [9]Department of Dermatology, Osaka University Graduate School of Medicine, Suita, [10]Department of Dermatology, Shimada Municipal Hospital, Shimada, [11]Department of Dermatology, Keio University School of Medicine, Tokyo, [12]Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, [13]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, [14]Department of Epidemiology and Environmental Health, Juntendo University Faculty of Medicine, Tokyo, [15]Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan, [16]Department of Dermatology, National Taiwan University College of Medicine, and [17]Department of Dermatology, Linkou Branch of Chang Gung Memorial Hospital, Taipei, Taiwan

ABSTRACT

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe adverse drug reaction caused by specific drug. It is characterized by visceral organ involvement and reactivation of various human herpesviruses. Although sporadic reports have documented certain conditions that appear after the resolution of DIHS/DRESS, little information is available on sequelae after resolution of DIHS/DRESS in a large patient population. The Asian Research Committee on Severe Cutaneous Adverse Reactions, comprised of doctors from Japan and Taiwan, conducted a survey on sequelae and deterioration of the underlying disease in patients with DIHS/DRESS. This was achieved by directly interviewing patients who had been followed-up by experts or through a questionnaire mailed to patients. Questions were asked about new onset cardiovascular disease, collagen disease or autoimmune disease, gastrointestinal disease, renal disease, respiratory disease, neoplasms, and other diseases such as herpes zoster and diabetes mellitus, as well as deterioration of the underlying disease. A total of 145 patients were analyzed in this study. The following newly developed diseases after recovery from DIHS/DRESS were observed: Graves' disease (*n* = 2), Hashimoto's disease (*n* = 3), painless thyroiditis (*n* = 2), fulminant type 1 diabetes mellitus (*n* = 5), and infectious diseases (*n* = 7). Several DIHS/DRESS patients with pre-existing renal dysfunction required lifelong hemodialysis. DIHS/DRESS is a condition that increases the risk of new onset of disease. Long-term observation of DIHS/DRESS can provide an opportunity to investigate substantial diseases from onset to the full-blown stage. Patients with DIHS/DRESS require careful long-term follow-up.

Key words: **autoimmune thyroiditis, drug reaction with eosinophilia and systemic symptoms, drug-induced hypersensitivity syndrome, fulminant type 1 diabetes mellitus, Graves' disease, Hashimoto's disease.**

Correspondence: Yoko Kano, M.D., Ph.D., Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka, Tokyo 181-8611, Japan. Email: kano@ks.kyorin-u.ac.jp

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INTRODUCTION

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe adverse drug reaction caused by specific drugs such as anticonvulsants and allopurinol. It is characterized by visceral organ involvement and reactivation of various human herpesviruses (HHV).[1–9] Sporadic reports have documented the appearance of newly developed diseases after the resolution of DIHS/DRESS, such as autoimmune thyroid disease,[10–13] type 1 diabetes mellitus,[14–17] sclerodermoid graft-versus-host disease-like lesions,[18] and systemic lupus erythematosus.[19] It is likely that DIHS/DRESS is a risk factor for triggering new onset of disease. The newly developed diseases could be recognized as sequelae of DIHS/DRESS. It is also likely that DIHS/DRESS is a risk factor for deterioration of pre-existing disease. However, little information is available on sequelae or deterioration of the underlying disease after resolution of DIHS/DRESS in a large patient population because of the difficulty of long-term follow-up after clinical resolution of DIHS/DRESS and the potential development of sequelae after a disease-free period of several months to years.[20,21] Despite this, it is important to clarify the association of DIHS/DRESS with the development of sequelae or deterioration of pre-existing disease. To investigate the link between DIHS/DRESS and the development of newly onset disease as suggested by previous reports, we surveyed patients from a total of 14 institutions in Japan and Taiwan, and analyzed the presence of sequelae and deterioration of the underlying disease in patients with DIHS/DRESS. Our findings suggest that late-onset complications, characteristic sequelae, and deterioration of pre-existing disease occur in patients with DIHS/DRESS.

PATIENTS AND METHODS

Patients with DIHS/DRESS who were treated in institutions belonging to the Japanese or the Taiwanese Research Committee on Severe Cutaneous Adverse Reaction (SCAR) between 1998 and 2013 were eligible for this study. All patients satisfied the diagnostic criteria for DIHS proposed by the Japanese SCAR group and/or the criteria for DRESS proposed by the international Registry of Severe Cutaneous Adverse Reactions.[22,23] The criteria for DIHS were the presence of a high-grade fever, widespread maculopapular and/or diffuse erythematous eruption, lymphadenopathy, leukocytosis with atypical lymphocytosis and/or eosinophilia, liver dysfunction, and HHV-6 reactivation. Because cases that satisfied the DIHS criteria were recognized as either definite or probable according to the Registry of Severe Cutaneous Adverse Reactions scoring system for DRESS,[24] DIHS and DRESS were recognized as homogenous conditions. The period of observation and follow-up of study patients was more than 6 months (range, 6 months–13 years; median, 4.9 years) after the onset of disease. There were a total of 215 DIHS/DRESS patients who were treated in participating hospitals. In addition to direct interviews conducted with the 44 patients who were regularly followed-up, including those without overt clinical or laboratory findings, a questionnaire was sent to 171 patients who were not undergoing regular follow-up (Fig. 1). The questionnaire asked about cardiovascular disease, collagen disease or autoimmune disease, gastrointestinal disease, ocular disease, renal disease, respiratory disease, tumor/cancer, and other diseases such as herpes zoster and diabetes mellitus (Table 1). Responses were obtained from a total of 158 patients. Patients who had died before initiation of the survey were excluded. This study was approved by the institutional review board of each participating institution.

RESULTS

Patient characteristics

The questionnaire response rate was 66.7%. Of the 145 DIHS/DRESS patients analyzed, 59 were men and 86 were women. The mean age at onset of DIHS/DRESS was 51.0 ± 18.8 years (range, 6–86 years). The culprit drugs were allopurinol, anticonvulsants (e.g. carbamazepine, phenobarbital, phenytoin, and zonisamide), antibiotics, mexiletine, and sulfa agents (e.g. diaphenylsulfone and salazosufapyridine). The underlying diseases treated by the causative drugs were arrhythmia, cerebral infarction, colitis, convulsion, encephalitis, epilepsy, hyperuricemia, immunoglobulin A nephritis, lupus erythematosus, neuralgia, psychiatric diseases, restless leg syndrome, rheumatoid arthritis, tonsillitis, and vasculitis. In the majority of patients, the culprit drug was discontinued when drug eruption was suspected. The causative drug was identified by the clinical course or using the lymphocyte transformation test and/or patch test. Most patients were treated by systemic corticosteroids, but some patients were managed with supportive therapy alone for dehydration. A 4–8-week treatment of oral corticosteroids was required to achieve complete resolution. In three patients, methylprednisolone pulse therapy (1000 mg/day for 3 days) was administered. One patient received plasmapheresis because of recurrence after systemic corticosteroid treatment. Cyclosporine was given to one patient. Some patients received topical corticosteroids for symptomatic relief.

Outcomes after DIHS/DRESS

Various newly developed diseases were documented after the resolution of DIHS/DRESS, including thyroid diseases, diabetes mellitus, herpes zoster, drug eruption, arthritis, pneumonia, thrombotic infarction, alopecia, systemic lupus erythematosus, and vitiligo (Table 2). Among these diseases, thyroid disease was the most frequent sequela in the present study. Seven of the 145 patients developed autoimmune thyroiditis after the onset of DIHS/DRESS. In patients with autoimmune thyroiditis, two had Graves' disease, three had Hashimoto's disease, and two had painless thyroiditis. Two patients had thyroid dysfunction without antithyroid antibodies. The age at onset of DIHS/DRESS was markedly younger in patients with Graves' disease (mean age, 30.0 years) than those with Hashimoto's disease (mean age, 67.0 years) and painless thyroiditis (mean age, 61.5 years). Five patients were women. Clinical manifestations

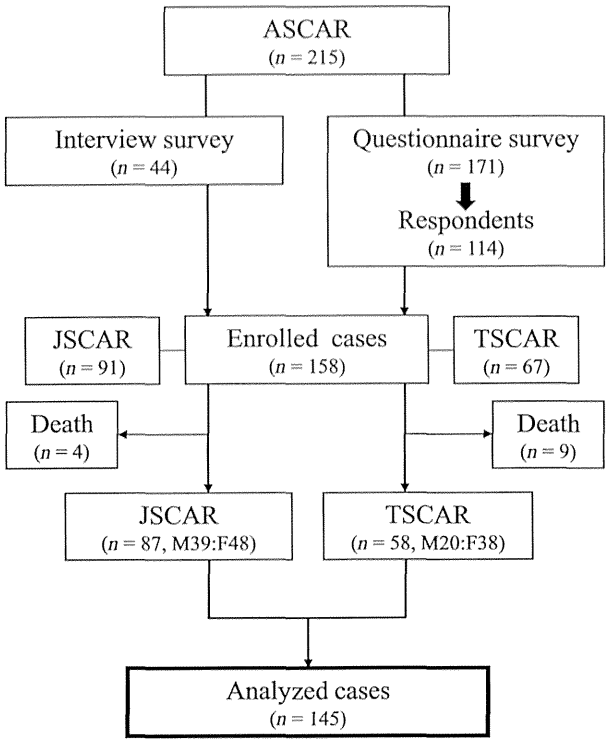

Figure 1. Patient flow diagram. ASCAR, Asian Research Committee on Severe Cutaneous Adverse Reaction; JSCAR, Japanese Research Committee on Severe Cutaneous Adverse Reaction; TSCAR, Taiwanese Research Committee on Severe Cutaneous Adverse Reaction.

Table 1. Interview questionnaire

Do you suffer from following diseases? Have you suffered from following diseases?

1. Ocular disease
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
2. Respiratory disease
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
3. Renal disease
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
4. Gastrointestinal disease
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
5. Cardiovascular disease
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
6. Collagen disease (Ex. Lupus erythematosus)
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible /☐No)
7. Tumor/Cancer (Ex. Lymphoma, gastric cancer)
   - ☐ No
   - ☐ Yes: Please tell the name of disease. _____
   (Relation to the drug eruption:☐Possible/☐No)
8. Other diseases
   - ☐ Herpes zoster
   - ☐ Thyroid disease
   - ☐ Type 1 diabetes mellitus/Type 2 diabetes mellitus
   - ☐ Drug eruption (Please tell the causative drug. _____
   - ☐ Others _____

that led to suspicion of autoimmune thyroiditis were alopecia, palpitation, and hand tremor in two patients with Graves' disease, and general fatigue in one patient with Hashimoto's disease. In the other two patients with Hashimoto's disease, the diagnosis was based on the results of follow-up laboratory examinations in the absence of overt clinical symptoms. In two patients with thyroid dysfunction alone, autoantibodies such as rheumatoid factor and antinuclear antibodies (ANA) were detected. The interval between onset of DIHS/DRESS and the appearance of autoimmune thyroiditis was 2 months to 3 years (Fig. 2).

Besides autoimmune thyroiditis, other autoimmune diseases and conditions such as alopecia, arthritis, systemic lupus erythematosus, and vitiligo were detected. Rheumatoid arthritis appeared with characteristic deformity of the joint more than 10 years after the onset of DIHS/DRESS, which had been managed with supportive therapy alone, and there was no family history of autoimmune disease. Vitiligo appeared in a female patient 4.5 months after the onset of DIHS/DRESS. In this patient, systemic corticosteroids had been given for DIHS/DRESS, but recurrence occurred after tapering the corticosteroids. Therefore, in addition to corticosteroids, cyclosporine was added.

Fulminant type 1 diabetes mellitus (FT1D) is a major concern during the follow-up of DIHS/DRESS because the abrupt onset of FT1D requires prompt intervention. In five patients with FT1D, the mean age at onset of DIHS/DRESS was 56.6 years (range, 21.0–84.0 years). No gender difference in the development of FT1D was observed in this study. FT1D developed within 2 months after the onset of DIHS/DRESS in all patients. The average interval between onset of DIHS/DRESS and the emergence of FT1D was 42.0 days (Fig. 3). Of these five patients, one was positive for anti-insulinoma-associated protein-2. Prompt intervention was initiated in all patients after the diagnosis of FT1D; therefore, no patients died from FT1D.

Table 2. Newly developed disease

Newly developed disease	Number of patients	Age or mean age (years)	Interval†	Published cases
Autoimmune thyroiditis				
Graves' disease	2 (M1:F1)	30.0	2 m, 9 m	Chen *et al.*[20]
Hashimoto's thyroiditis	3 (F)	67.0	6 m–3 yr	Ushigome *et al.*[21]
Painless thyroid disease	2 (M1:F1)	61.5	2 m, 2 yr	
Thyroid dysfunction††	2 (F)	53.0	1 m, NA	
DM				
Fulminant type 1 DM	5 (M3:F2)	56.6	1–2 m	Chiou *et al.*[16], Chen *et al.*[20]
Type 2 DM	1 (F)	64	3 m	
Herpes zoster	5 (M3:F2)	59.6	2 m–3 yr	Ushigome *et al.*[21], Kano *et al.*[26]
Drug eruption	4 (M2:F2)	60.5	2–6 yr	Ushigome *et al.*[21]
Arthritis				
Reactive arthritis	1 (F)	63	3 m	Morito *et al.*[25]
Rheumatoid arthritis	1 (F)	48	10 yr	
Arthralgia	1 (F)	67	11 m	
Pneumonia	2 (M)	70.5	8 m, 1.5 yr	Ushigome *et al.*[21]
Thrombotic infarction‡	2 (M)	63.5	2 m	Hashizume *et al.*[27]
Alopecia§	1 (F)	45	4 m	Ushigome *et al.*[21]
Systemic lupus erythematosus¶	1 (M)	36	3.5 yr	Aota *et al.*[19]
Vitiligo	1 (F)	45	4.5 m	

†Between the onset of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, and the detection of newly developed diseases. ‡Inferior vena cava and cerebral blood vessel, respectively. §No thyroid disease. ¶After the onset of subacute necrotizing lymphadenitis. ††Thyroid dysfunction cannot be included as autoimmune thyroiditis because it may develop as a prior condition. Therefore, this is in parentheses.DM, diabetes mellitus; F, female; M, male; m, month; NA, not available; yr, year.

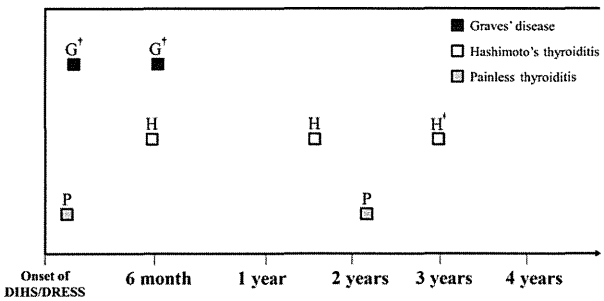

Figure 2. Detection of autoimmune thyroiditis. DIHS/DRESS, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms; G, Graves' disease; H, Hashimoto's thyroiditis; P, painless thyroiditis; †Reported by Chen *et al.*[20] ‡Reported by Ushigome *et al.*[21]

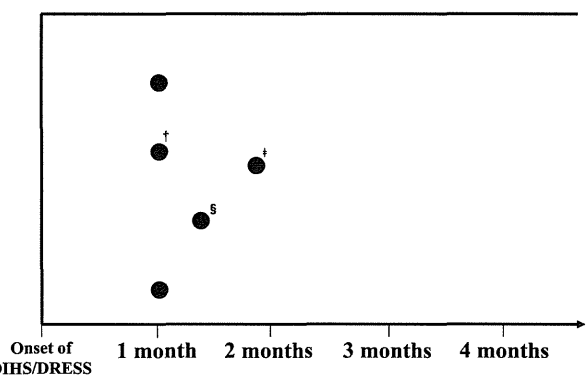

Figure 3. Onset of fulminant type 1 diabetes mellitus. DIHS/DRESS, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. †Anti-insulinoma-associated protein-2 was detected. ‡Reported by Chiou *et al.*[16] §Reported by Chen *et al.*[20]

In most patients, DIHS/DRESS was treated with systemic corticosteroids.

Several manifestations related to viral reactivations were detected in this study. Herpes zoster appeared in five patients. Herpes zoster lesions developed within 3.5 months after the onset of DIHS/DRESS in three of the five patients; they developed 3 years after onset in one patient; and the interval was unclear in the other. Infarction in a cerebral lesion and a limb was documented in one patient each approximately 2 months after the onset of DIHS/DRESS. Both patients were diagnosed with thrombotic infarction, and cytomegalovirus reactivation was detected in this period in both. Pneumonia was detected in two patients, and the infectious agent was *Cryptococcus* in one patient and undetermined in the other. Some of the cases described above have been previously described elsewhere.[19–21,25–27]

Alterations in the underlying disease were observed as a result of the commencement of hemodialysis (HD) in patients with renal disease, autoimmune hemolytic anemia in a patient with systemic lupus erythematosus, and resolution of restless leg syndrome. With respect to deterioration of the underlying disease, four patients with renal disease required lifelong HD. The mean age at onset of DIHS/DRESS in these four patients was 55.0 years (range, 24.0–79.0 years). The causative drugs of DIHS/DRESS were allopurinol (n = 2) and diaphenylsulfone

(n = 1); the causative drug was unknown in one patient. The interval between the onset of DIHS/DRESS and commencement of HD ranged from 0.5 month to 5 years. Even a young patient with immunoglobulin A nephritis developed irreversible renal insufficiency after DIHS/DRESS. Autoimmune hemolytic anemia occurred in a patient with systemic lupus erythematosus. Surprisingly, the recalcitrant symptoms of restless leg syndrome disappeared 3 months after the onset of DIHS/DRESS; DIHS/DRESS had been managed with supportive therapy alone in this patient (Table 3).

DISCUSSION

The development of sequelae such as autoimmune thyroiditis and FT1D after several months or years has been described in many reports.[10–17] However, previous similar studies with a small sample size may have been affected by sampling bias and thus might not be representative of the outcome of DIHS/DRESS. In this study, the relatively larger number of patients from 14 institutions in two countries provided findings that are more reliable than those of previous reports. In addition, DIHS/DRESS was diagnosed by experts on drug reactions in this study. Therefore, the evaluation of patients was accurate. The present study revealed that DIHS/DRESS could lead to the occurrence of various sequelae, many of which may have been overlooked had the follow-up survey of patients not been performed by dermatologists and other experts. However, this survey does have a limitation: patients who have a newly developed disease or who have manifested clinical symptoms tend to respond to this kind of medical questionnaire. Furthermore, the follow-up intervals of each patient were not defined in order to obtain short- and long-term sequelae. Therefore, the diverse differences in observation periods among patients precluded comparisons between incidences of newly developed diseases in this survey and those in the general population. In addition, family history, detailed laboratory analysis and viral reactivation, and detailed treatment were not analyzed.

In this study, various newly developed diseases after DIHS/DRESS were documented. They include autoimmune diseases and autoimmune-related diseases, FT1D, and infectious diseases. With regard to autoimmune diseases, six patients (four from Japan and two from Taiwan) developed autoimmune diseases, such as autoimmune thyroiditis, reactive arthritis, and systemic lupus erythematosus, after recovery from DIHS/DRESS;[19–21,25] these patients were included in the present study. In this survey, autoimmune thyroiditis, including Graves' disease, Hashimoto's disease and painless thyroiditis, was the most common disease after recovery from DIHS/DRESS, with a prevalence of 4.8% (7/145). Together with previous studies,[10–13,18] the present results suggest an association between DIHS/DRESS and the appearance of autoimmune thyroiditis. A female predominance in patients with autoimmune thyroiditis after DIHS/DRESS was similar to that observed in the general population. Graves' disease was detected in patients who were younger than those with other autoimmune thyroid diseases such as Hashimoto's disease and painless thyroiditis, a trend similar to that observed in the general population. The interval between the onset of DIHS/DRESS and autoimmune thyroiditis ranged from 2 months to 2 years. In view of our previous study showing that autoantibodies such as antithyroid peroxidase and antithyroglobulin antibodies were detected without any clinical manifestations of thyroiditis after the clinical resolution of DIHS/DRESS,[21] it is likely that the production of antithyroid antibodies might precede the clinical appearance of autoimmune thyroiditis in patients with DIHS/DRESS. Considering that autoimmune thyroiditis, in particular Hashimoto's disease, has been frequently linked to genetic background, family history should have been examined in this survey.

Brown *et al.* documented the coexistence of autoimmune thyroiditis and autoimmune FT1D in a patient with DRESS. In this case, various autoantibodies, including anti-glutamic acid decarboxylase, antithyroid peroxidase, antithyroglobulin, ANA, and anti-Sjögren's syndrome A, were detected.[11] Therefore, the possibility of overlapping autoimmune diseases was raised. In addition, a recent report described the concurrent development of FT1D and Hashimoto's disease at the onset of DIHS/DRESS, characterized by the presence of antithyroglobulin antibodies, ANA, and anti-Sjögren's syndrome A antibodies with the absence of glutamic acid decarboxylase and islet cell antibodies.[28] In the present study, a case of rheumatoid arthri-

Table 3. Alteration of underlying disease

Alteration of underlying disease	Underlying disease	Number of patient (M:F)	Age or mean age (years)	Interval†	Published cases
Onset					
Autoimmune hemolytic anemia	SLE	1 (F)	35	2 m	Chen *et al.*[20]
Deterioration					
Induction of hemodialysis	CRI	2 (M1:F1)	65.5	0.5 m, 2.5 yr	Chen *et al.*[20]
	IgA nephritis	1 (M)	24	5 yr	
	Renal disease	1 (F)	65	1 yr	
Resolution					
Symptoms	Restless leg syndrome	1 (M)	72	3 m	

†Between the onset of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, and alteration of underlying disease. CRI, chronic renal insufficiency; IgA, immunoglobulin A; m, month; SLE, systemic lupus erythematosus; yr, year.

tis was seen. In this patient, the appearance of autoimmune antibodies, such as antithyroid peroxidase, antithyroglobulin antibodies, and ANA, was observed without any clinical symptoms 3 years after the onset of DIHS/DRESS, and bone deformity developed 10 years later, after the disappearance of these antithyroid antibodies. These findings indicate that several autoimmune diseases can occur concurrently or sequentially in patients with DIHS/DRESS.

It is unclear why autoimmune diseases develop in patients with DIHS/DRESS. Considering the viral involvement in the development of autoimmune diseases, several articles have reported that herpesvirus infections might contribute to the occurrence of autoimmune thyroiditis. Descamps suggested a possible association between HHV-6 reactivation and autoimmune thyroid disease because the presence of HHV-6 in the thyroid was significantly higher in Hashimoto's thyroiditis than in controls.[29] Based on the observed discrepancy between the viral reactivation period and the onset of autoimmune thyroiditis, the host immune response may also play a pivotal role in the appearance of autoimmune thyroid disease. From an immunological perspective, our previous study showed that the number of fully functional $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells is markedly increased in the acute stage of DIHS/DRESS compared with other drug reactions, which contributed to viral reactivation. These Treg cells lost their ability to inhibit cytokine production and proliferation of effector T cells, which coincided with their contraction upon clinical resolution of DIHS/DRESS.[9,30,31] This functional defect of Treg cells might be responsible for the emergence of autoimmunity. In addition, it is likely that drug eruption in four patients after recovery from DIHS/DRESS might be associated with this functional defect of Treg cells.

FT1D is a subtype of diabetes mellitus characterized by an abrupt onset, absence of islet-related autoantibodies, and nearly complete destruction of pancreatic β-cells. FT1D and autoimmune type 1 diabetes mellitus have been linked to DIHS/DRESS.[11,14–17] In particular, many articles reported that FT1D can occur in association with DIHS/DRESS.[11,14–17] Although one patient had islet cell antibodies in the present study, all diagnosed cases had features that were compatible with FT1D. In this current survey, the prevalence of FT1D was 3.45% (5/145). Previous reports and our present results strongly suggest that DIHS/DRESS could trigger the development of FT1D. The mean interval between the onset of DIHS/DRESS and FT1D was 42.0 days in the present study. This interval was comparable with the finding of an interval of 39.9 days in a previous article.[17] Although we are unable to provide a satisfactory explanation for the development of FT1D, a strong association between HLA-B62 and FT1D in Japanese patients with mexiletine-induced DIHS/DRESS has been demonstrated.[17] Based on this finding, it is worthwhile to investigate the contribution of genetic susceptibility to the development of FT1D on a large scale, including in Taiwanese patients with DIHS/DRESS. Factors that predict the development of FT1D were not found in this study.

Infectious diseases such as herpes zoster and cryptococcal pneumonia were observed after the resolution of DIHS/DRESS.[21,26] Accumulating evidence suggests that various herpesviruses reactivate during the course of DIHS/DRESS, but varicella zoster virus reactivations have rarely been reported during the course of the disease. Because herpes zoster is frequently observed without any relationship to the underlying disease, it is very difficult to determine whether there is any association between herpes zoster and the preceding DIHS/DRESS. However, considering that two patients developed herpes zoster after dose reduction of systemic corticosteroids,[26] herpes zoster is likely one of the manifestations of immune reconstruction inflammatory syndrome in the setting of DIHS/DRESS.[32] The occurrence of cryptococcal pneumonia might also be regarded as a manifestation of the immune reconstruction syndrome in this setting. Interestingly, two patients had thrombotic infarction at the same time, approximately 2 months after the onset of DIHS/DRESS.[27] Given that reactivation of cytomegalovirus was detected at this time and the characteristic intranuclear inclusion body of cytomegalovirus is frequently observed in endothelial cells,[33] it is likely that the onset of thrombotic disease in the two patients was not coincidental but might have been caused by cytomegalovirus reactivation. It seems that these conditions might have been overlooked in previous cases of DIHS/DRESS.

Four patients with pre-existing renal dysfunction due to chronic renal insufficiency and immunoglobulin A nephritis required HD within 5 years after the onset of DIHS/DRESS. Although it is extremely difficult to determine whether deterioration was related to the prior occurrence of DIHS/DRESS, DIHS/DRESS could increase the risk of progression to renal failure in the setting of prior renal function disturbance. Further special attention needs to be given to this possibility.

In conclusion, our results indicate that DIHS/DRESS might contribute to the new onset of diseases after recovery from DIHS/DRESS. DIHS/DRESS is a condition that provides an invaluable opportunity to observe newly developed disease from their initiation to the full-blown stage. Patients with DIHS/DRESS require careful long-term follow-up.

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CORRESPONDENCE

Differential expression profile of Th1/Th2-associated chemokines characterizes Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) as distinct entities

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) are two of a triad of severe cutaneous adverse reactions (SCAR) to drugs [1, 2]. The rapid recognition of DIHS/DRESS and SJS/TEN is essential because they are potentially life-threatening syndromes. Thus, diagnostic markers or predictive factors need to be defined.

We previously reported that thymus and activation-regulated chemokine (TARC/CCL17) serum levels were markedly higher in the acute stage of DIHS/DRESS than in other forms of drug eruption [3, 4]. We also demonstrated that TARC levels in the acute stage of DIHS/DRESS correlated with disease activity [3]. In this study, we attempted to identify chemokine patterns that would allow us to distinguish between the different forms of drug eruptions and gain insight into the pathomechanisms involved.

We first examined the expression of macrophage-derived chemokine (MDC/CCL22), a chemokine related to TARC, in patients with DIHS/DRESS, SJS/TEN and maculopapular exanthema (MPE). A previous study showed that the CC chemokines, TARC and MDC, were T helper (Th) 2-associated chemokines that bind to CC chemokine receptor 4 (CCR4) on Th2 cells [5]. We subsequently compared the expression of the Th1-associated chemokines, monokine induced by IFN-γ (MIG/CXCL9) and IFN-inducible protein 10 (IP-10/CXCL10). MIG and IP-10 are related chemokines of the CXC subfamily that are known to share the receptor, CXCR3, on Th1 cells [5]. Blood samples were obtained from 11 patients with DIHS/DRESS, 18 patients with SJS/TEN and 15 patients with MPE in the acute stage and after recovery; serum concentrations of TARC, MDC, IP-10, and MIG were measured by ELISA. Diagnosis of SJS/TEN and DIHS was made on the basis of the criteria proposed by Auquier-Dunant *et al* [6] and Shiohara *et al* [7], respectively. The results obtained revealed marked differences between the different types of SCAR. In addition to an increase in TARC (*figure 1A*), which is consistent with the findings of our previous study [3, 4], the expression of MDC was markedly higher in DIHS/DRESS than in the other forms of drug eruption (*figure 1A*). In contrast to DIHS/DRESS, the expression of the Th1-associated chemokines (MIG and IP-10) was higher in SJS/TEN than in MPE (*figure 1A*). These results were consistent with the findings of previous studies in which SJS/TEN was characterized by a predominantly Th1 pattern of activation [8, 9]. Our results clearly showed slightly higher MIG and IP-10 values in SJS/TEN than in DIHS/DRESS but statistical significance was not reached between these two groups, which indicated that DIHS/DRESS also exhibited a Th1 pattern of activation to some extent. The relatively small number of patients and the overlap in the range distribution in DIHS/DRESS *vs* SJS/TEN and DIHS/DRESS *vs* MPE imply that further extended studies are needed to reach a concrete conclusion.

We then investigated whether the levels of the upregulated chemokines described above declined upon remission. Serum levels of MDC in DIHS/DRESS and MIG and IP-10 in SJS/TEN decreased in the remission stage (*figure 1B*), which was also observed in TARC in DIHS/DRESS (*figure 1B*) [3].

A recent study reported that biopsy specimens from SJS/TEN cutaneous lesions exhibited a mixed Th1/Th2 pattern [10]. The same group subsequently demonstrated that TARC levels were significantly higher in the sera of SJS/TEN patients than in the sera of healthy donors (HD) [9]. The median TARC levels reported were 580 pg/mL in SJS/TEN and 205.2 pg/mL in HD [9]. Our previous studies also showed elevated levels of TARC in SJS/TEN patients; average TARC levels were 2,198 pg/mL in one study [3] and 1,543 pg/mL in the other [4] (normal value of TARC: <450 pg/mL). However, our present study did not confirm this finding. Although further evidence is needed for a clear conclusion, the markedly lower TARC values observed in SJS/TEN than in DIHS/DRESS suggest that a Th1 response may play a major role in SJS/TEN with the minimal coexistence of a Th2 response, if any.

Taken together, our results showed that Th2-associated chemokines were markedly upregulated in DIHS/DRESS, while Th1-associated chemokines predominated in SJS/TEN. This result indicates that the mechanisms

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