書　籍

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	論文タイトル	第 2 章 他科（皮膚科・形成外科）による重症度評価が必要な皮膚障害. 分子標的薬を中心とした皮膚障害.						
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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)

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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.**

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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

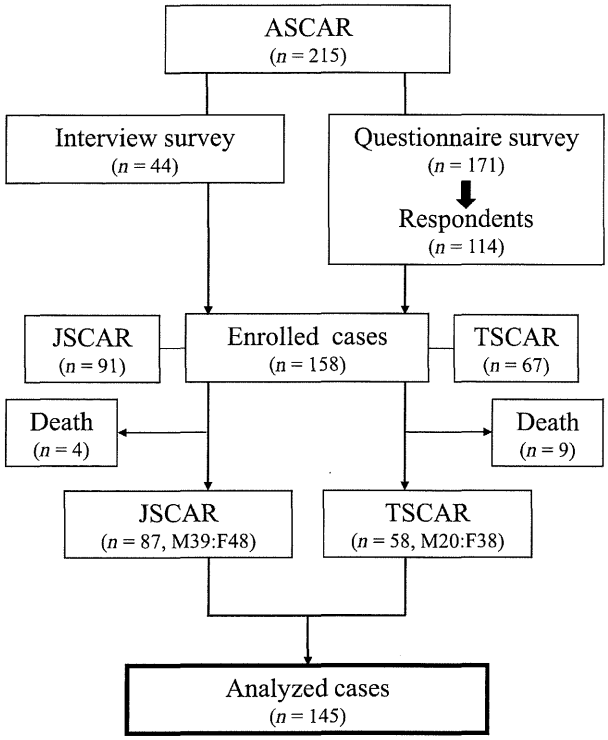

Figure 1. Patient flow diagram. ASCAR, Asian Research Committee on Severe Cutaneous Adverse Reaction; JSCAR, Japanese Research Committee on Severe Cutaneous Adverse Reaction; TSCAR, Taiwanese Research Committee on Severe Cutaneous Adverse Reaction.

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.

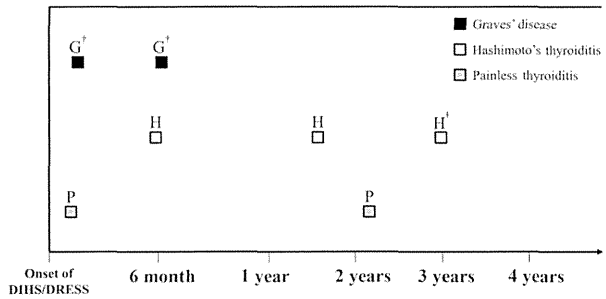

Figure 2. Detection of autoimmune thyroiditis. DIHS/DRESS, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms; G, Graves' disease; H, Hashimoto's thyroiditis; P, painless thyroiditis; †Reported by Chen *et al.*[20] ‡Reported by Ushigome *et al.*[21]

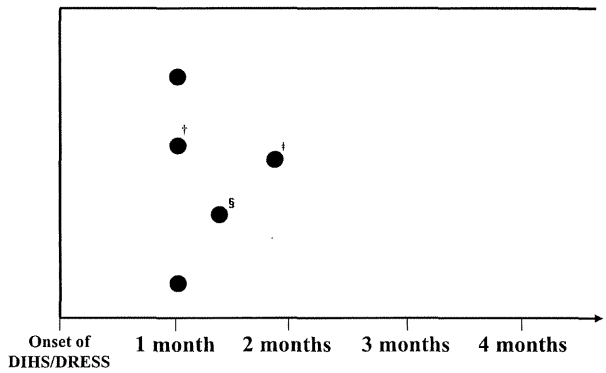

Figure 3. Onset of fulminant type 1 diabetes mellitus. DIHS/DRESS, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. †Anti-insulinoma-associated protein-2 was detected. ‡Reported by Chiou *et al.*[16] §Reported by Chen *et al.*[20]

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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Regulatory T Cells in Severe Drug Eruptions

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Abstract: Regulatory T cells (Tregs) are essential for limiting immunopathology and maintaining immune homeostasis. They represent a major barrier to aberrant and excessive immune responses to pathogens and allergens in infectious and allergic diseases, respectively. In this review, we describe our current understanding of the immunopathogenic mechanism behind protection against the development and exacerbation of severe drug eruptions, with special emphasis on regulatory T cells (Tregs). In this regard, our previous study demonstrated that the timing of the dysfunction of Tregs could determine the pathological phenotype and sequelae of severe drug eruptions. We also discuss the factors that abrogate Treg function and demonstrate that *Mycoplasma pneumoniae* is the only pathogen shown to cause a persistent loss of Treg function long after clinical resolution. A loss of Treg function observed at the different stages of severe drug eruptions would be a driving force in the subsequent development of autoimmune disease as long-term sequelae of severe drug eruptions.

Keywords: Drug-induced hypersensitivity syndrome, fixed drug eruption, herpesviruses, *Mycoplasma pneumoniae*, regulatory T cells, Stevens-Johnson syndrome, toxic epidermal necrolysis.

INTRODUCTION

Organs that are exposed to external environment, such as the skin and gastrointestinal tract, possess physical and biochemical barriers to microbial and chemical challenges and mechanical injury [1], and have evolved a variety of strategies to control inflammation and maintain immunological homeostasis. Although the skin is the primary organ targeted by the host immune response to drug, a delicate balance between the ability to react with some drugs and the ability not to react with other drugs is necessary to ensure the integrity of skin tissue. However, factors involved in disrupting this equilibrium existing in the skin are largely unknown, although both genetic and environmental factors have been implicated [2-5]. In this regard, viral and mycoplasmal infections are recognized as risk factors for the development of drug eruptions and as a major inducer of exacerbation of drug eruptions [6-8]. Immune responses in the context of such infections can thus have varying effects on the potential development of allergic inflammation [9], especially drug eruptions. Indeed, available evidence also strongly suggests that viral and mycoplasmal infections create a favorable milieu for the initiation and progression of adverse drug eruptions [10, 11], although the mechanisms whereby preceding viral or mycoplasmal infections induce or contribute to the development of drug eruptions are currently unknown. On the other hand, in order to maintain or restore a homeostatic environment, the anti-viral or anti-mycoplasmal immune responses could paradoxically create an environment that protects the host from excessive immune responses to these pathogens and allergens, which could in itself lead to greater pathological consequences than the invading pathogens and allergens themselves. Thus, generation and the subsequent activation of protective T cell responses that can ameliorate immune response-associated inflammation are a key component of immune reactions to drug.

$Foxp3^+CD4^+$ regulatory T cells (Tregs) represent a developmentally and functionally distinct T cell subpopulation that can suppress such aberrant and excessive immune responses [12, 13]. Evidence is recently accumulating that Tregs, either natural or induced, can inhibit the function of T effector cells (Teffs) at the site of microbial infections and allergic inflammation, thereby inhibiting severe immunopathology [14]. On the other hand, the Treg response is potentially harmful to the host in terms of infection control [15], because their activation may secure survival of invading pathogens for an extended period of time, thereby causing chronic infectious diseases. Numbers and functions of Tregs, therefore, should be controlled depending on the stage of infections and inflammation.

In this review, we describe our current understanding of the immunopathogenic mechanisms behind protection against the development and exacerbation of drug eruptions, with particular reference to severe drug eruptions, Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (DiHS/DRESS).

Tregs in SJS/TEN

Because the most prevalent severe eruptions are thought to be mediated by drug-specific Teffs, the phenotype and functions of these Teffs are likely to determine the clinical picture of the disease. Alternatively, however, severe drug eruptions could be induced by a disbalance of the immune system caused by excessive activation of Teffs associated with an inadequately low function or number of Tregs that can limit immunopathology. In support of this possibility, Azukizawa *et al.* [16, 17] reported that in an animal model of TEN Tregs can prevent experimentally induced epidermal injury mimicking

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TEN, although the therapeutic effect cannot be observed. Their studies suggested the importance of Tregs in protecting susceptible patients from the development of TEN. Thus, drug-induced immunopathology in SJS/TEN is likely to be subject to control by Tregs. However, the degree to which Tregs truly played a role during the actual disease process in protecting the host's tissues from severe immunopathology in SJS/TEN was largely unknown.

The frequency of Tregs in total circulating $CD4^+$ T cells from patients with SJS/TEN at the acute stage was not significantly different from that in those from healthy controls [18]. Previous studies demonstrated that Tregs in healthy controls preferentially express chemokine receptors and adhesion molecules required for skin homing, such as CLA and CCR4 [19, 20]. This skin-homing phenotype was also found in Tregs in patients with SJS/TEN, although the frequency was slightly lower than healthy controls. In contrast, our immunohistochemical study showed that $Foxp3^+$ Tregs were rarely found in skin lesions, where multiple blisters developed: these results suggested their functional defects in patients with SJS/TEN. To examine their functional activity on a per-cell basis, we performed CD3-driven T-cell proliferation assay by coculturing FACS-sorted $CD4^+CD25^-$ Teffs with FACS-sorted $CD4^+CD25^{++}$ Tregs at different Tregs:Teffs ratios. Tregs obtained from patients with SJS/TEN at the acute stage were found to be profoundly defective in their capacity to suppress T cell proliferation (Fig. 1). The degree of functional defect was directly related to the severity of epidermal damage. Nevertheless, their defective capacity at the acute stage was returned to the presumed baseline of the patient before the onset of SJS/TEN upon clinical resolution [18]. These findings suggest the functionality of Tregs at the acute stage of SJS/TEN was impaired on a per-cell basis in agreement with our observation that severe epidermal damage can be seen in skin lesions of SJS/TEN patients, in whom circulating Tregs were present in normal frequency.

Could a functional defect in Treg recruitment into the inflammatory site accounts for the exacerbated pathology in SJS/TEN? To resolve this issue, we have to ask whether the skin immune system could limit the duration or intensity of the inflammatory response would rely on the capacity to direct Tregs to the site of action. We therefore compared the acute skin lesions of SJS/TEN with the corresponding lesions of a generalized bullous variant of fixed drug eruptions (gbFDE), whose clinical symptoms at the acute stage are indistinguishable from SJS/TEN. Despite such clinical similarities between gbFDE and SJS/TEN, subsequent evolution of the two conditions is quite different: the former resolves spontaneously upon discontinuation of the causative drug whereas the latter often results in full-thickness epidermal detachment, rapidly spreading to the whole body. Because the individual erythematous lesions in SJS/TEN form poorly defined macules rapidly extending to the perilesional uninvolved skin while the FDE lesions usually have well-defined border, the defect in regulatory mechanisms for preventing further disease progression to SJS/TEN could reside either within the cutaneous milieu in the inflammatory site, particularly in the periphery or within migrating Tregs themselves. Circulating Tregs obtained from gbFDE patients preferentially expressed CLA, CCR4 and CCR6 as demonstrated in healthy controls. The percentages of $CCR4^+$ cells and $CCR6^+$ cells in total $Foxp3^+$ Tregs were significantly lower in peripheral blood mononuclear cells (PBMCs) of SJS/TEN patients than those in PBMCs in gbFDE patients and healthy control. In addition, Tregs obtained from gbFDE patients were found to retain the suppressive capacity to inhibit CD3-driven proliferation of Teffs. Importantly, our immunohistochemical study on acute skin lesions of gbFDE and SJS/TEN showed that gbFDE lesions, especially in the periphery, were characterized by increased frequency of Tregs as compared with the corresponding SJS/TEN lesions. Particularly when the ratio

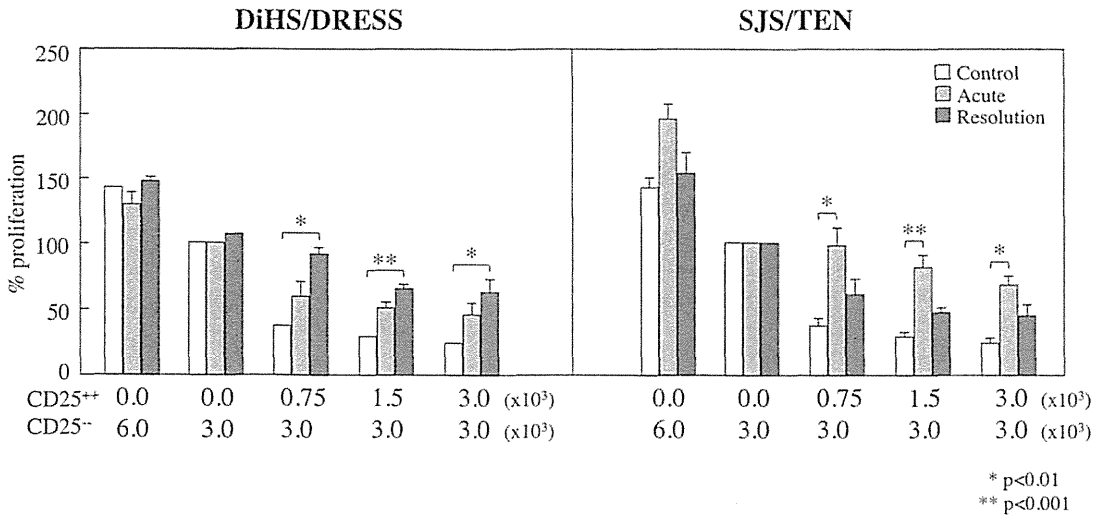

Fig. (1). Functional analysis of Tregs at the different stages of DiHS/DRESS and SJS/TEN. Highly purified $CD4^+CD25^{++}$ Tregs from patients with DiHS/DRESS or SJS/TEN or healthy controls were cocultured at different ratios with highly purified $CD4^+CD25^-$ Teffs from the same individuals in the presence of mitomycin C-treated allogeneic APCs and CD3 and CD28 mAbs. The results are expressed as the percent proliferation of Teffs in the absence of Tregs. (Modified from our previous study (18))[§].

[§]Mizukawa Y. *et al.* Mast cells contribute to preferential migration of regulatory T cells through the release of IL-16 in fixed drug eruption lesion. Manuscript in preparation

of Tregs to $CD8^+$ T cells was determined in individual samples, the ratio was the highest in the epidermis of the periphery of gbFDE lesions with the corresponding area of SJS/TEN lesions being the lowest (Mizukawa Y, *et al.* manuscript in preparation). Of note, $Foxp3^+$ Tregs preferentially accumulated beneath the epidermis and at the mid part of the dermis in the periphery of the gbFDE lesions, while those were sparsely distributed in the upper part of the dermis of the periphery of SJS/TEN lesions. These findings can be interpreted as indicating that timely and selective migration of Tregs into the periphery of gbFDE lesions could be crucial for preventing the excessive activation and recruitment of $CD8^+$ Teffs (Fig. 2). Our ongoing experiments clearly have suggested that mast cells abundantly residing in gbFDE lesions may facilitate such rapid and timely recruitment of Tregs to the inflammatory site although impaired recruitment of Tregs to SJS/TEN lesions could not solely be ascribed to low frequencies of mast cells. Thus, Treg recruitment to the inflammatory site could be controlled by local tissue-dependent factors, such as mast cells resident in the site, rather than an absolute, intrinsic homing ability of Tregs. Based on our data, IL-16 would be the key mast-cell-produced cytokine that can trigger Treg recruitment to the inflammatory site in gbFDE lesions, although IL-16 is not the only cytokine to have such functions [21, 22]. A rapid and proper localization of Tregs into the specific inflammatory site would serve to limit activation of potentially destructive $CD8^+$ Teffs, resulting in spontaneous resolution of gbFDE lesions upon withdrawal of the causative drug.

Treg Function in Infections with *Mycoplasma pneumoniae*

Mycoplasma pneumoniae (MP), a member of the smallest wall-less bacterial class, occurs as commensals or pathogens in animals and humans: *MP* is one of the most common causes of atypical pneumonia in pediatric and adult populations worldwide, while it causes asymptomatic infection in most humans [23, 24]. Although it can infect multiple organ systems, cutaneous symptoms can be seen in 20 to 25% of patients [25], some of which are maculopapular eruption and self-limited; however, more serious complications of *MP* infection, such as SJS/TEN, often occur both during and after active infection, although the mechanisms whereby *MP* infection might induce or contribute to the development of severe life-threatening drug eruptions in susceptible individuals are currently unknown. The main difficulty in assigning a pathogenic role to *MP* in these severe drug eruptions is that the majority of immunocompetent individuals infected with *MP* are asymptomatic and the infection will go unrecognized unless a serologic search is made to identify *MP*. We therefore explored the hypothesis that frequencies and function of Tregs could be specifically altered by *MP* infection depending on the stage of infection.

We evaluated the frequencies of Tregs in total PBMCs of patients with *MP* infection and those with other viral infection, such as varicella zoster virus (VZV) and parvovirus B19 (B19) infections, at their acute and resolution stages, respectively. No significant alterations, in the mean frequencies of Tregs were found in these patiens at

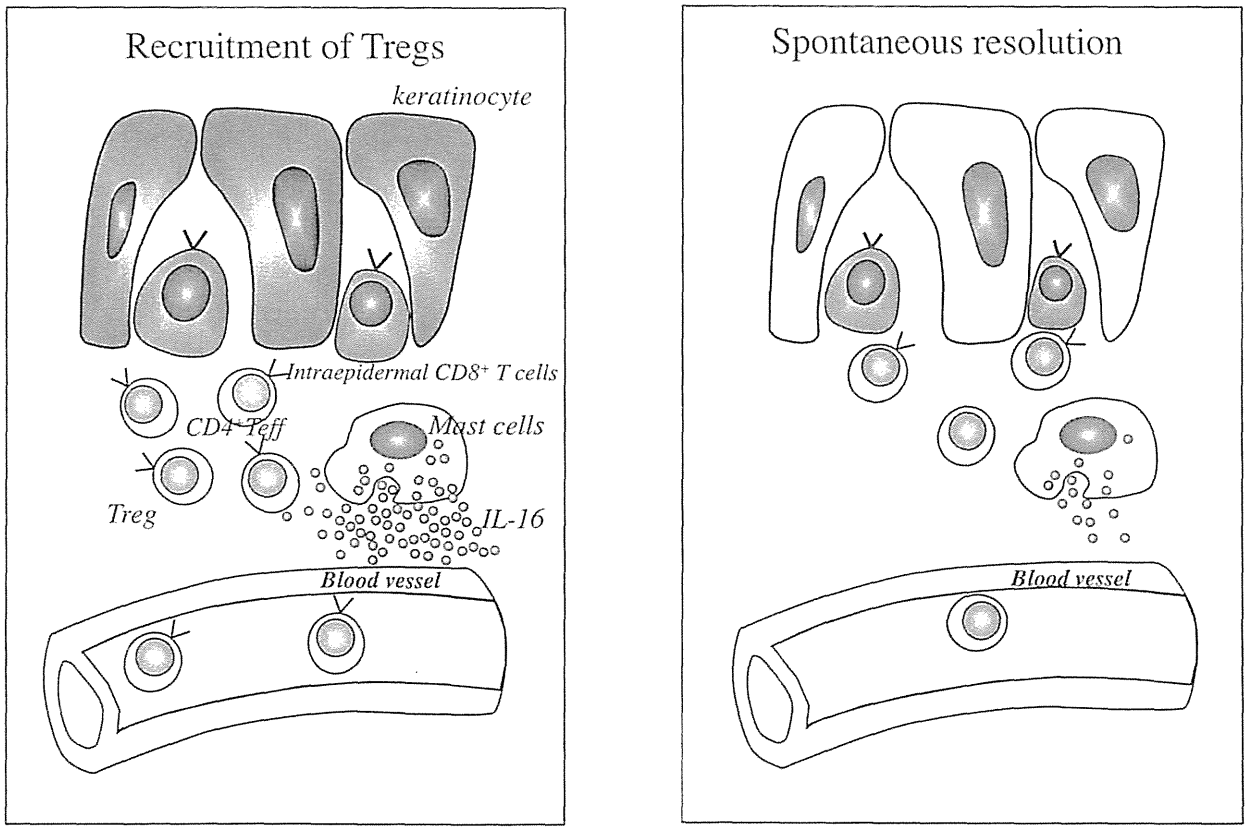

Fig. (2). Spontaneous resolution of FDE lesions induced by Treg recruitment to the inflammatory site, upon withdrawal of the causative drug.

all the time points examined, as compared with those of age-matched healthy controls. Patients with mild respiratory symptoms and slight erythema who were positive for *MP* IgM and showed a significant increase in PA and CF titers were enrolled in our study. Patients with primary VZV or B19 infection were also enrolled as those with viral infections. In humans, CCR6 expression on Tregs has been shown to reflect their functional migratory properties [26]. We therefore asked whether $CCR6^+$ Tregs could be altered during acute infection with *MP* or viruses. Frequencies of $CCR6^+$ Tregs were significantly decreased in patients with *MP* infection, regardless of the stage examined as compared with those in patients with viral infections and healthy controls (unpublished data).

We next examined whether the Tregs obtained from patients with *MP* infection are functionally defective by coculturing FACS-sorted $CD4^+CD25^-$ Teffs with FACS-sorted $CD4^+CD25^{++}$ Tregs obtained from the same patients. Tregs obtained from these patients with acute infection, either *MP* or viruses, exhibited a significantly impaired capacity to suppress CD3-driven, Teff proliferation, as compared with those from healthy controls (Fig. 3). The degree of functional defect in patients with acute viral and mycoplasmal infection was comparable to that in patients with SJS/TEN (Takahashi R, *et al.* Manuscript in preparation). Their impaired capacity at the acute stage of viral infection, however, had returned to a presumed baseline, which was indistinguishable from that of healthy controls, upon resolution. In contrast, functional activity of Tregs obtained from *MP* patients remained defective even after clinical resolution. Surprisingly, the impairment in suppressive function of Tregs remained detected even 1 year after clinical resolution, although the magnitude of the impairment became gradually less apparent (Fig. 3): this result was somewhat different from that observed in SJS/TEN, in which functional activity of Tregs was returned to the presumed baseline of the patient. These contradictory results suggest that there is more to be learned about the functional impairment of Tregs in the setting of SJS/TEN and MP infection not associated with SJS/TEN. We infer that systemic corticosteroids we used for the treatment of SJS/TEN would have served to rapidly restore the impaired function of Tregs because none of patients with MP infection had been treated with systemic corticosteroids. We also found that $CD4^+CD25^-$ Teffs from either the acute or resolution stage of *MP* infection were not resistant to suppression by Tregs from healthy controls, indicating that the defect in *MP* infection resides in the Tregs than in the Teffs (Takahashi R *et al.* Manuscript in preparation).

Thus, our study clearly demonstrates that *MP* infection persistently abrogates Treg function for an extended period of time, a finding never observed in infections with other pathogens including viruses. In view of the actions of Tregs, it makes good biological sense that a temporal limitation in Treg function or number is usually associated with subsequent better control of the acute infection by enhancing immune responses to the pathogens because suppression of the early immune response to infection would be harmful to the host. Nevertheless, restoration of Treg function is likely to occur at later time points in infections. Thus, a time-dependent balanced, rather than biased, Treg responses would be necessary for host protection and the resolution of infection. To date, as far as we tested, *MP* is the only pathogen shown to cause a persistent loss of Treg function even long after clinical resolution, while in other viral infections the defective Tregs regain their functional

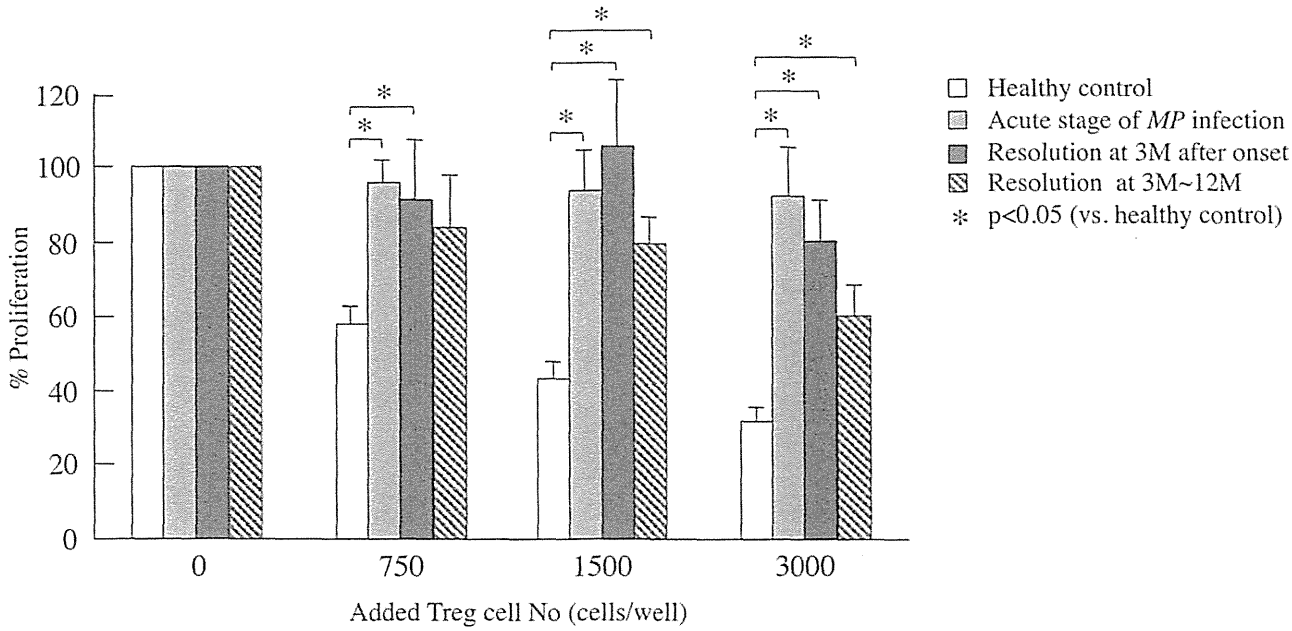

Fig. (3). Functional analysis of Tregs in patients with *MP* infections at the various time points after onset. Experiments are performed as described in the legend of Fig 1: graded numbers of Tregs as shown were added to Teffs°.

°Takahashi R. *et al.* Mycoplasma infection persistently abrogates the function of regulatory T cells. Manuscript in preparation.

competence upon clinical resolution. We can hypothesize that decreasing Treg function in patients with *MP* infection would serve to lower the activation threshold of drug-specific T cells, thus facilitating the development of drug eruptions. Although it remains unknown how *MP* infection persistently abrogates Treg function, ligation of Toll-like receptors (TLRs) by *MP* may be responsible for a persistent loss of Treg function. In this regard, recent studies have shown that ligation of TLR2 on Tregs by synthetic ligands temporarily abrogates their suppressive function [27] and that stimulation of 1α, 25VitD3-induced IL-10-secreting human Tregs with TLR9 ligands results in a loss of Treg function [28]. Given the ability of *MP* to stimulate TLR2 and 4 signaling pathway [29, 30], Tregs are likely to lose their suppressive capacity in response to engagement of TLRs.

Expansions of Tregs in DiHS/DRESS

DiHS/DRESS and SJS/TEN represent the opposite ends of a spectrum of severe drug eruptions. DiHS/DRESS offers a unique opportunity to elucidate the mechanism by which viral infections could affect the development of severe drug eruption, because previous studies established its strong association with human herpesvirus 6 (HHV-6) infection [31, 32]. This syndrome has several unique features that cannot be solely explained by a drug Ag-driven oligoclonal T-cell activation: the delayed onset in relation to the introduction of the causative drug is one of the important features of this syndrome that can be distinguished from other types of drug eruptions, which usually start 1-2 weeks after starting therapy: this syndrome typically occurs with fever and cutaneous lesions 3 weeks to 3 months after starting therapy with a limited number of drugs, mainly anticonvulsants [31-34]. Importantly, more severe reactions often occur 3-4 days after withdrawal of the causative drugs: this paradoxical worsening is also characteristic of DiHS/DERSS and may be erroneously labeled as severe infectious diseases. In addition, variable clinical symptoms, such as renal and liver symptoms, continue to deteriorate one after another even weeks or months after stopping the causative drug. Most erythematous macules do not evolve into blisters and no mucous membrane involvement is usually seen [33-35].

Despite such variable clinical presentations and courses, HHV-6 reactivations can be detected at a particular time point, 2-3 weeks after onset of rash in the vast majority of patients regardless of treatment [31, 33, 35]: a strong association between the magnitude of HHV-6 reactivations and the severity of this syndrome has been supported by a large number of independent groups over years in Japan [34, 36]. Recent studies of real-time measurements for viral loads during the course of DiHS/DRESS have demonstrated that not only HHV-6 but also other herpes viruses, such as Epstein-Barr virus (EBV), HHV-7 and cytomegalovirus (CMV), are reactivated in sequence as demonstrated in graft-versus-host disease (GVHD) [37]: the cascade of reactivation events initiated by HHV-6 or EBV would extend, with some delay, to HHV-7 as well, and eventually to CMV. These findings provide strong evidence to suggest the role of herpes viruses in the etiology of the disease, rather than a mere bystander, although reactivation of these viruses as a result of a transient immune dysfunction cannot be definitely excluded. Thus, immune systems of patients with DiHS/DRESS are characterized by inadequate control of herpes virus replication and highly variable waxing and waning nature of generalized immune activation.

Although it has been suggested that DiHS/DRESS is caused by an exaggerated cellular immune responses to either drugs, reactivated viruses or both [38], a link between sequential occurrence of herpes virus reactivations and Tregs has not been convincingly established. Given their potent suppressive capability, the role of Tregs in the clinical course of DiHS/DRESS could be either harmful or beneficial: they could be postulated to have a negative impact on herpes viruses infection by suppressing efficient anti-herpes virus-specific immune responses on the one hand, while they could be beneficial to the host by dampening excessive self-inflicted immune activation triggered by viruses or drug on the other. Their role would be different depending on the

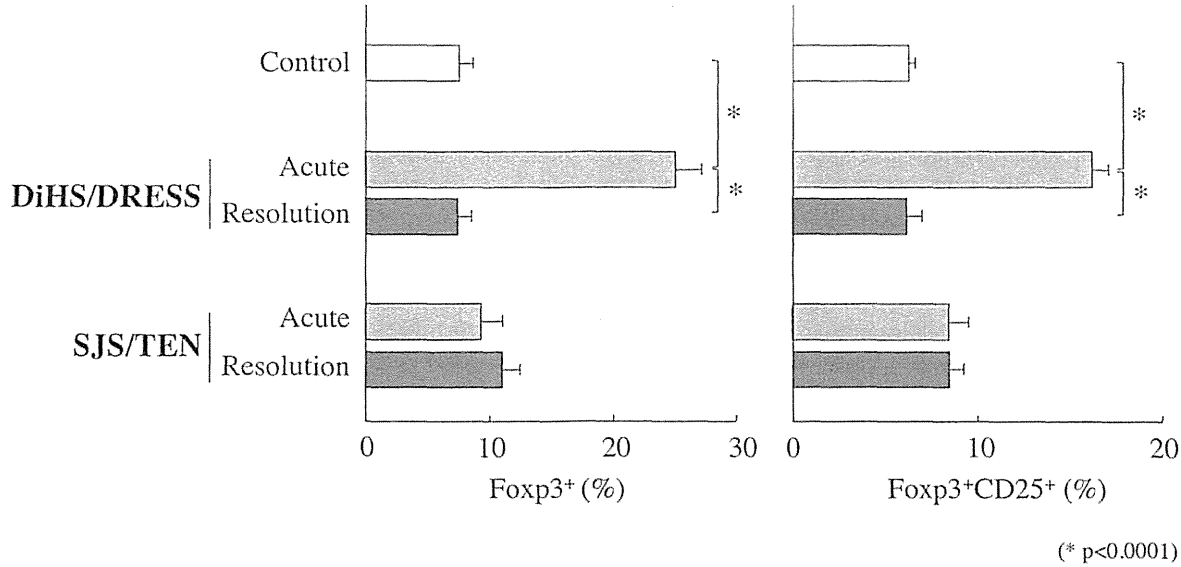

Fig. (4). Expansions of circulating Foxp3$^+$ Tregs at the acute stage of DiHS/DRESS but not of SJS/TEN. (Modified from our previous study [18]).

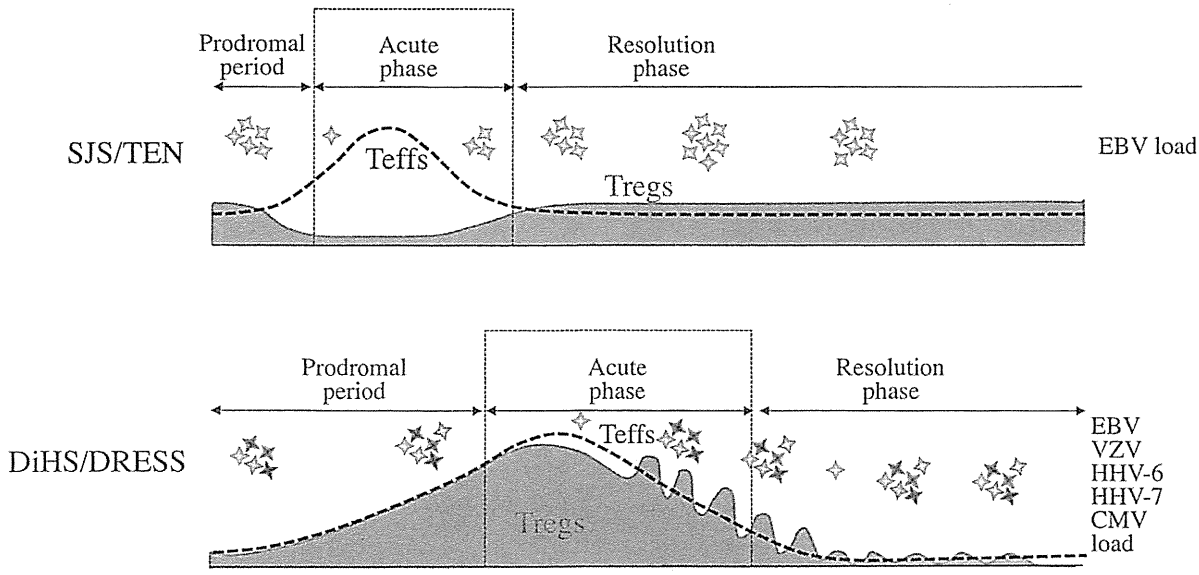

Fig. (5). Hypothetical model for the relationship among Tregs, Teffs and viral reactivation patterns in two types of severe drug eruptions. Systemic corticosteroids used for treatment of these severe drug eruption would serve to restore the functional impairment of Tregs.

stage of disease, the compartments in which Tregs are examined, and viral loads. According to our recent study [18], the acute stage of DiHS/DRESS is characterized by dramatic expansions of fully functional Tregs: the relative percentage of Tregs in circulating $CD4^+$ T cells was dramatically increased during the acute stage of DiHS/DRESS as compared with that in healthy controls (Fig. **4**). Although it is difficult to precisely determine when Treg expansions occur during the course of disease, this expansion would occur far before onset of DiHS/DRESS, which would contribute to not only the delayed onset but also viral reactivation [34]. In order to counterbalance activating Teffs, expansions of Tregs are likely to be key for maintaining a healthy balance between protection and immunopathology. However, once the balance has been disturbed toward activation of Teffs, DiHS/DRESS ensues (Fig. **5**). Thus, the expanded Tregs would also limit the severity of Teff-mediated immunopathology, which is reflected by the observation that epidermal damage can be rarely detected in the skin lesions of DiHS/DRESS.

A recent study [39] has indicated that Tregs can be classified into functionally distinct subpopulations based on CD45RA and Foxp3 expression levels: $CD45RA^+Foxp3^+$ resting/natural occurring Tregs (rTregs) and $CD45RA^-Foxp3^{++}$ activated/induced Tregs (iTregs), both of which have suppressive function *in vitro*, and $CD45RA^-Foxp3^+$ nonsuppressive T cells (non-Tregs) (Fig. **6A**). We therefore investigated whether Tregs expanded during the acute stage of DiHS/DRESS could represent the $CD45RA^-Foxp3^{++}$ iTregs phenotype. Although non-Tregs were also increased in the acute stage of DiHS/DRESS, iTregs were dramatically increased in the acute stage as compared with those in healthy controls (Fig. **6B**). This finding indicates that expanded Tregs in the acute stage of DiHS/DRESS are of the iTreg phenotype and that they could be induced in the periphery under specific conditions of cytokines and antigen, because iTregs can be produced from $CD4^+CD25^-$ T cells by culture with antigen and TGF-β or IL-10 and TGF-β, while IL-6 inhibits iTregs induction and promotes Th17 [40, 41]. Consistent with this view, our preliminary study shows that *in vitro* culture of PBMCs from DiHS/DRESS patients after resolution with the causative drug results in expansions of iTregs. In view of our observation that there is an overall increase in the total number of $CD4^+$ T cells in blood, this increase in Tregs during the acute stage of DiHS/DRESS could be actually much more than that in the relative percentage of Tregs. In addition, Tregs expanded in patients with DiHS/DRESS at the acute stage were found to retain the suppressive capacity indistinguishable from those in healthy controls [18]. Importantly, because not only Tregs but also Teffs are expanded during the acute stage of DiHS/DRESS (unpublished observation), it remains to be determined whether expanded Tregs indeed play a critical role for sequential reactivation of herpesviruses by blunting the immune responses to these herpesviruses. Further studies investigating cohorts followed up longitudinally would be required to address the precise role of Tregs in the modulation of herpesvirus-associated immune activation.

Thus, expansions of iTregs with immunosuppressive function provide potential mechanisms by which herpesviruse could be reactivated during the course of DiHS/DRESS. However, no satisfactory explanation for why only herpesviruses can be reactivated in sequence has been available; but some clues may come from studies of $CD16^+$ monocytes, which have been shown to mediate epidermal damage in SJS/TEN [42]. In contrast, they are not involved in the pathogenesis of DiHS/DRESS. Thus, monocyte migration to the inflammatory skin sites is likely the key to progression to SJS/TEN, but not to DiHS/DRESS. Indeed, because blood monocytes are shown to be major effectors involved in the innate responses to viral infections [43], their functional or numerical defects would leave the host vulnerable to severe viral infections. Human blood monocytes are heterogeneous populations and can be separated into three distinct subsets on

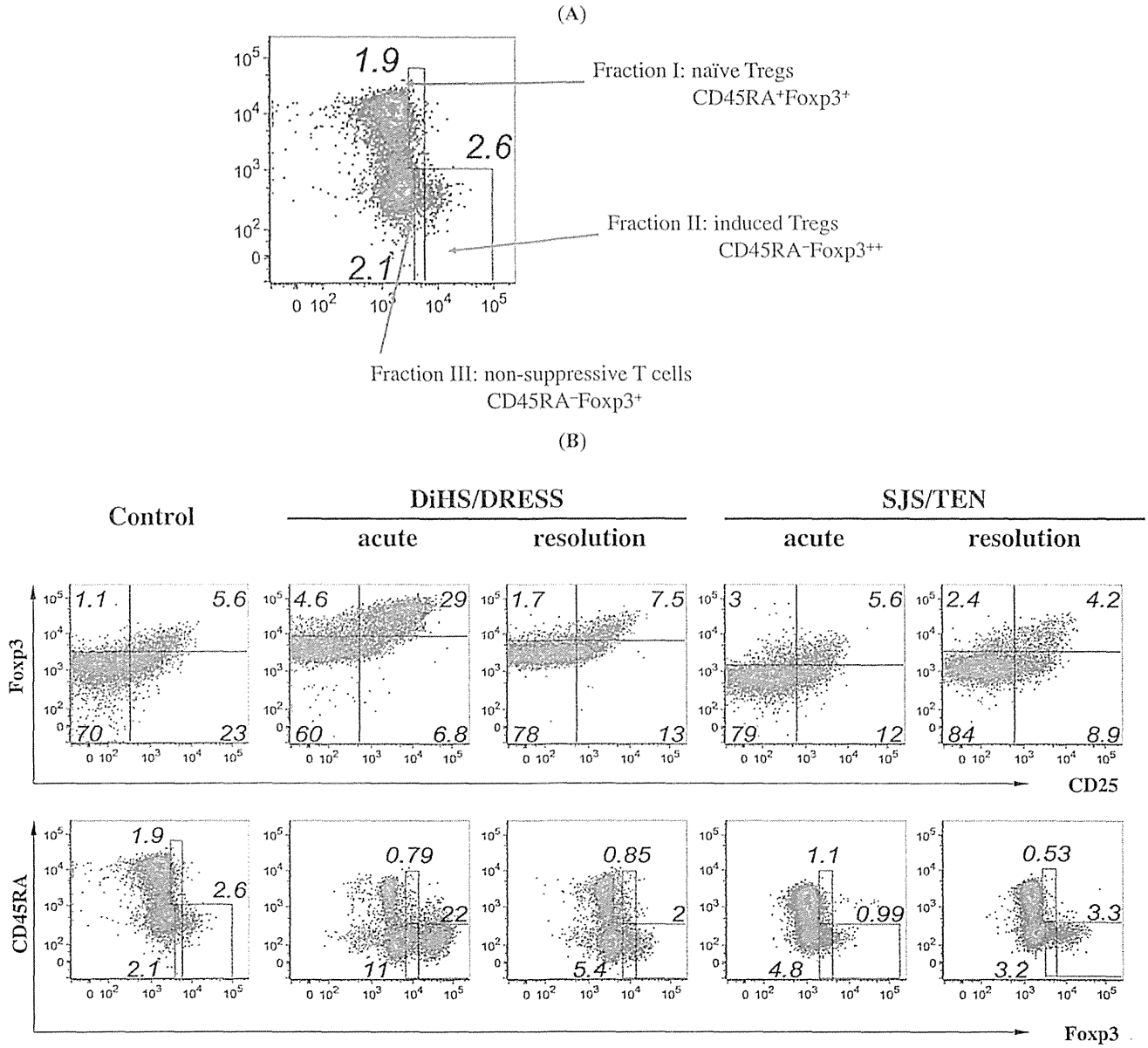

Fig. (6). Three functionally distinct subpopulations of Tregs. **A**. Three fractions in healthy controls. **B**. Expansions of Treg FrII in the acute stage of DiHS/DRESS.

the basis of their phenotypical and functional features: $CD14^{++}CD16^{-}$ classical monocytes (cMOs), $CD14^{+}CD16^{+}$ intermediate monocytes (iMOs), and $CD14^{dim}CD16^{+}$ nonclassical, proinflammatory monocytes (pMOs) [43, 44]. Among them, $CD14^{dim}CD16^{+}$ monocytes have received increasing attention over the last 4 years, because this population has been shown to patrol blood vessels and selectively detect virally infected cells to produce proinflammatory cytokines, TNF-α, IL-1β and CCL3, thus mediating anti-viral roles [43, 44]. These findings suggested that this population, pMOs, would be numerically or functionally impaired in patients with DiHS/DRESS who cannot control viral reactivations. We therefore investigated the dynamics of monocyte subsets in relation to those of Tregs in DiHS/DRESS and SJS/TEN. Surprisingly, pMOs have been depleted from the circulation and skin lesions in the acute stage of DiHS/DRESS, while the corresponding skin lesions of SJS/TEN were characterized by massive infiltrations of pMOs. This preferential depletion of pMOs was associated with expansions of Tregs in DiHS/DRESS: there was an inverse relationship between pMOs and Tregs (Fig. 7). More importantly, paired immunoglobulin-like type 2 receptor a (PILR-α) and herpesvirus entry mediator (HVEM), which can specifically bind to herpes simplex virus (HSV) envelope glycoprotein B (gB) and gD, respectively [45, 46], were preferentially expressed on pMOs. Because expansions of Tregs are only observed in the acute stage of DiHS/DRESS but not in SJS/TEN, it is logical to ask whether such alterations in monocyte subsets could be responsible for driving iTreg expansions in DiHS/DRESS. Our ongoing studies clearly show that cMOs have the most efficient capability to expand iTregs while pMOs have much less capability: consistent with the

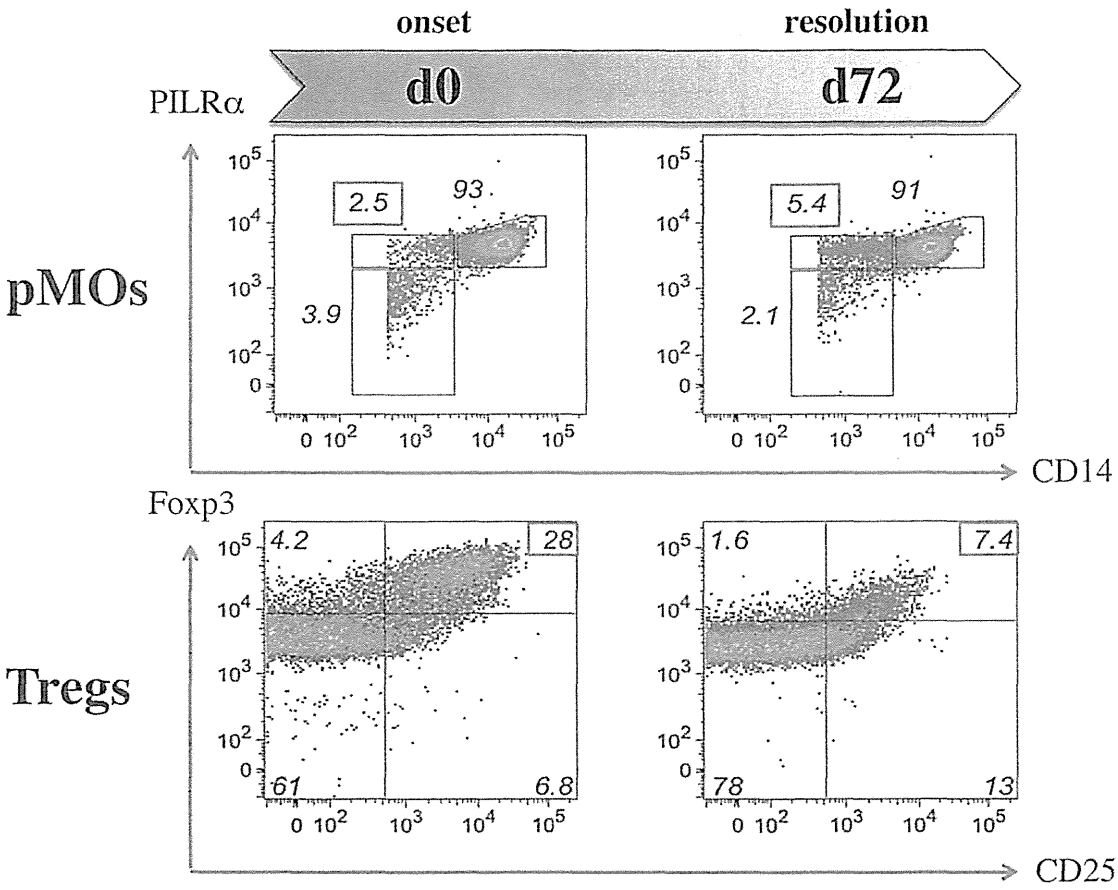

Fig. (7). Expansions of Tregs inversely associated with preferential depletion of pMOs expressing PILR-α during the clinical course of DiHS/DRESS.

result, a recent work has revealed that pMOs control the proliferation of Tregs in immune thrombocytopenia [47]. Our CFSE-based assays demonstrate that pMO-depleted MOs have the most potent capability to induce iTreg expansions and that *in vitro* stimulation of PBMCs from DiHS/DRESS patients with the causative drug induced selective proliferation of iTregs specific for drug (unpublished observation). These results indicate that preferential depletion of pMOs could function as a driving force behind expansions of Tregs and subsequent herpesvirus reactivations, eventually resulting in clinical disease (Fig. 7). This dynamic interaction between Tregs and MOs inferred from our data may be critical to initiation and exacerbation of a pre-existing anti-drug immune responses.

The role of Tregs in Autoimmune Sequelae of DiHS/DRESS

Viruses have been proposed repeatedly as triggering factors for development of autoimmune disease. However, a major difficulty in establishing a correlation between triggering viral infections and the actual autoimmune disease is that during the long prodromal period preceding the clinical onset of disease the virus involved at the early stage would have been eliminated by an anti-viral immune response, thereby making its direct identification at the lesion difficult later on when autoimmune disease has developed. In this regard, DiHS/DRESS is an excellent disease in which to directly observe the course of disease from viral reactivations to development of autoimmunity. Indeed, our observation study in our University hospital over ten years found a prevalence of autoantibodies, such as anti-nuclear antibody (ANA) and thyroglobulin antibody (Tg Ab), or autoimmune diseases in ~10% of patients with DiHS/DRESS [48]. In addition, our recent longitudinal analysis of Treg function during the acute stage and long after clinical resolution has demonstrated that size of Treg population contracts upon resolution of DiHS/DRESS and the remaining cells become functionally impaired over a prolonged period of time [18]: such a gradual loss of Treg function after resolution would be a driving force in the subsequent development of autoimmune disease. In contrast, although onset of SJS/TEN was associated with a functional defect of Tregs, this functional defect was eventually restored after clinical resolution.

We therefore asked whether serum autoAbs against epidermal proteins including periplakin could be detected in samples obtained at various time points including those during the acute stage and long after clinical resolution of SJS/TEN and DiHS/DRESS. Previous studies reported that sera obtained from the acute stage of SJS/TEN and erythema multiforme contain autoAbs against epidermal proteins which could be generated as a consequence of epidermal damage [49]; in the study, it has been suggested that these autoAbs might be involved during the process of epidermal

damage. In this regard, however, our recent studies clearly have shown that the existence of these autoAbs was not restricted to patients with SJS/TEN but was extended to those with DiHS/DRESS characterized by no epidermal damage. More importantly, these autoAbs were present in these patients beyond the time frame of the acute inflammatory response, particularly in patients with DiHS/DRESS. In some patients with DiHS/DRESS these autoAbs levels appeared to gradually increase with time. A likely interpretation of these findings is that the generation of autoAbs is neither a direct consequence of severe epidermal damage nor a primary cause of epidermal damage at least in patients with DiHS/DRESS. Because Treg function is severely impaired in the acute stage of SJS/TEN and after resolution of DiHS/DRESS, respectively, the defective Treg responses observed in the different stage of these drug eruptions provide an explanation for why autoimmune responses can be generated during the course of the disease of SJS/TEN and after clinical resolution of DiHS/DRESS [50], respectively.

CONCLUSION

We propose a model for the pathogenesis of severe drug eruptions that involves Tregs and MOs. With regard to the immune mechanisms, however, that drive the loss of Tregs or pMOs before, during, or after the development of severe drug eruptions, very little is known in patients, because the disease process would begin days or weeks before the development of clinically apparent eruptions. Therapies in severe drug eruptions should try to target the generation and maintenance of Tregs, particularly iTregs, to mediate complete resolution of the disease during very early disease development: however, the mechanisms they use to exert their suppressive function differ depending on the disease phenotype, stage and inflammatory status of the local environment. Further studies are needed to determine whether several therapeutic strategies currently used for the treatment of severe drug eruptions could maintain or enhance Treg function.

CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare.

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