occurs after resolution of TEN.[22] Dry skin and heat intolerance are common complaints among survivors of SJS/TEN with involvement of the eccrine duct, although the secretory gland is usually normal.[23]

The sudden appearance of numerous melanocytic nevi following severe bullous lesions in a patient with SJS had been reported; it was speculated that the production of cytokines and growth factors during epidermal regeneration may have led to the proliferation of melanocytes.[24] In a separate case, widespread eruption of ectopic sebaceous glands occurred 4 months after an episode of SJS.[25] Similar to the aforementioned case, cytokines and growth factors were thought to be responsible for the proliferation of residual sebaceous gland cells.[25]

Pulmonary sequelae in SJS/TEN

The incidence of pulmonary involvement in SJS/TEN has not been examined. Pulmonary involvement in SJS/TEN is divided into two types: interstitial pneumonia during the course of SJS/TEN, and obliterative bronchitis/bronchiolitis after the resolution of SJS/TEN.[26] According to one report, pulmonary sequelae tend to occur in relatively young patients.[27] A few cases of obliterative bronchitis/bronchiolitis after SJS/TEN have been documented, as well as respiratory tract obstruction and bronchiectasis. The interval from the onset of SJS/TEN to development of pulmonary sequela is unclear because some reported cases show persistent respiratory symptoms from the onset of SJS/TEN.[27]

Obliterative bronchitis/bronchiolitis is diagnosed using imaging and respiratory function tests, with findings of bronchiectasis on high resolution computed tomography (CT) of the chest, occlusion of the bronchus on bronchoscopy, and a severe obstructive pattern in the flow–volume curve. Although the pathomechanism remains unknown, immunological pathways, infection, and remodeling of the bronchial mucosa are implicated in the pulmonary sequelae of SJS/TEN.

If patients suffer from recurrent respiratory symptoms after the resolution of SJS/TEN, they should be closely monitored using respiratory function tests and CT. No effective treatment is available for permanent obstructive pulmonary changes in obliterative bronchitis/bronchiolitis. In severe cases, mechanical ventilation is required, and living-donor lung transplantation may be necessary. Patients with pulmonary sequela after resolution of SJS/TEN tend to have a poor prognosis.

Esophageal sequelae in SJS/TEN

Long-term sequelae involving the gastrointestinal tract have rarely been reported. Esophageal stricture as a consequence of SJS has been reported in children but is rare in adults.[28,29] Two patients had foreign bodies lodged in esophageal strictures, occurring at 7 months after the episode of SJS in one case and at 18 months after the episode of SJS in the other case. The delay in the onset of dysphagia suggests that stricture formation may be subclinical. SJS/TEN-related esophageal stricture is thought to occur because of irritation caused by orally administrated medications, ingestion of coarse food, or nasogastric feeding during SJS/TEN. Esophageal strictures after SJS/TEN are easily dilated, suggesting that the condition is caused by injury to the esophageal mucosa without involvement of the muscularis.[29]

Sequelae in DIHS/DRESS

DIHS/DRESS is a severe adverse drug reaction caused by specific drugs such as anticonvulsants and allopurinol, and is characterized by visceral involvement and reactivation of human herpesviruses (HHV).[30–32] Various internal organs can be affected during the course of disease.[33–39] Furthermore, the development of autoimmune diseases several months to years after clinical resolution of DIHS/DRESS have been reported.[2,3] Several autoimmune diseases can develop sequentially in a single patient.[40] The emergence of autoimmune disease might be overlooked unless dermatologists perform long-term follow-up of patients after their recovery from DIHS/DRESS (Figure 3).[41]

Internal organ failure

Previous reports reveal that severe renal insufficiency increases the risk of mortality, and mortality depends in part on the degree of renal involvement rather than hepatic involvement.[41] Renal insufficiency following after acute interstitial nephritis at the acute stage of DIHS/DRESS could require a lifetime hemodialysis.[2] It is more likely to occur in elderly patients with pre-existing renal disease or those receiving diuretic therapy. Because renal function declines steadily with age, elderly patients are vulnerable to renal complications and sequelae.

Appearance of autoantibody after onset of DIHS/DRESS

Autoantibodies have been detected in patients with DIHS/DRESS after resolution of the disease. According to an analysis of 34 cases of DIHS/DRESS at our institution, autoantibodies such as antinuclear antibody (ANA), anti-thyroperoxidase (TPO), and anti-thyroglobulin antibodies were observed without any clinical manifestations.[3] The proportion of DIHS/DRESS patients with autoantibodies is higher than that seen in SJS/TEN patients.[42] The percentage of DIHS/DRESS patients with autoantibodies was higher in the group that received supportive treatment alone than those who received systemic corticosteroids.[3] The autoantibody titers fluctuated but remained elevated during the observation period. Our study showed that autoantibodies are present in some patients after clinical resolution of DIHS/DRESS without causing clinically overt diseases.

Autoimmune thyroid disease as a sequela in DIHS/DRESS

Thyroid disease is the most frequently detected sequela following the resolution of DIHS/DRESS, with a cumulative incidence of 3.8%. This incidence is more than 10-fold higher than the expected incidence of this disease in the Chinese population.[2] Endocrinologic evaluation of patients with DIHS/DRESS revealed thyroid gland abnormalities, such as increased free thyroxine (FT4), low thyroid-stimulating hormone (TSH), and elevated TSH levels, and production of autoantibodies, including anti-TSH receptor, anti-TPO, and anti-thyroglobulin antibodies. As symptoms are not usually observed, these abnormal findings may be missed if evaluation of thyroid function and antibodies are not performed.

Graves' disease may develop after the resolution of DIHS/DRESS. Recently, it has been reported that the interval between discontinuation of the causative drug and the onset of Graves' disease is approximately 2 months to 1 year. A recent case report described the appearance of a diffuse large thyroid goiter followed by hyperthyroidism 2 months after the onset of sulfasalazine-induced DIHS/DRESS.[43] Thyrotoxicosis can be the initial presenting symptom of thyroid disease.[44] Two patients diagnosed with Graves' disease with symptoms of hyperthyroidism and elevation in FT4 plus suppression of TSH have been documented—one case had symptoms 1 month after the onset of DIHS/DRESS while the other had symptoms 9 months after the onset of DIHS/DRESS.[2] Brown et al described a patient with Graves' disease confirmed by thyroid tests that developed 5 months after withdrawal of the causative

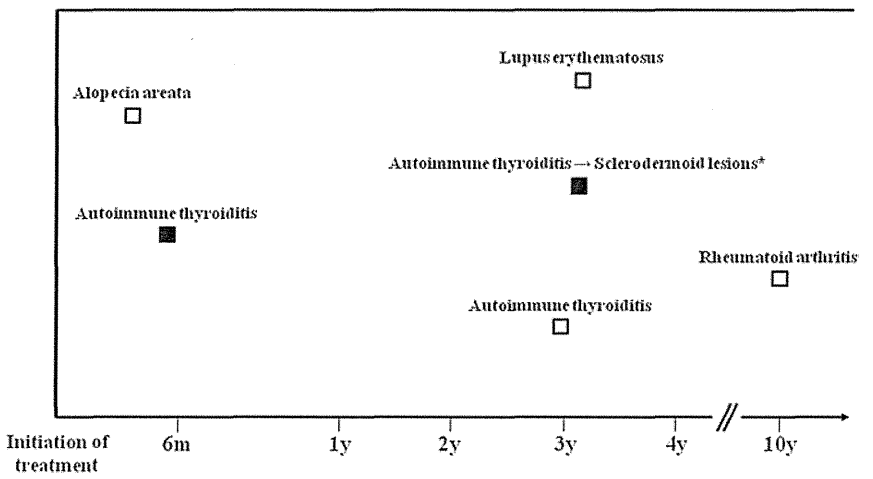

Figure 3 Autoimmune diseases developed after resolution of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). Six out of the 37 patients with DIHS/DRESS developed autoimmune diseases in our institution. The period of follow-up was more than 6 months after the initiation of treatment. (■), treated with systemic corticosteroid; (□), treated with supportive therapy alone; *, sclerodermoid graft-versus-host disease (GVHD)-like lesions.

drug.[45] The patient had low TSH and elevation of FT4 without symptoms of hyperthyroidism, and tests for anti-TSH receptor antibody and thyroid-stimulating immunoglobulin were negative at that time. Autoimmune thyroiditis can develop following the relapse of DIHS/DRESS.[46]

The emergence of Hashimoto's disease, characterized by the presence of anti-TPO antibody and anti-thyroglobulin antibodies, has also been observed after the resolution of DIHS/DRESS. The authors have encountered a patient with DIHS/DRESS who developed Hashimoto's disease 3 years after resolution of the clinical symptoms of DIHS/DRESS, characterized by elevated levels of anti-TPO and anti-thyroglobulin antibodies.[3] Alopecia has been noted in patients with autoimmune thyroid disease.[2,3,40] The presence of HHV-6 in the thyroid was significantly higher in Hashimoto's thyroiditis than in controls, highlighting a possible association between HHV-6 reactivation and autoimmune thyroid disease.[47,48]

Type 1 diabetes mellitus as a sequela in DIHS/DRESS

Some cases of fulminant type 1 diabetes mellitus (FT1D) have been associated with DIHS/DRESS.[49–52] FT1D is a subtype of type 1 diabetes mellitus (T1D) characterized by an abrupt onset, absence of islet-related autoantibodies, and nearly complete destruction of pancreatic β-cells. The initial symptoms of T1D are vomiting and dull epigastric pain; laboratory examinations reveal hyperglycemia, hyperosmolarity and metabolic acidosis, and a relatively normal glycosylated hemoglobin level; these features are compatible with diabetic ketoacidosis. The onset of FT1D is characterized by elevated levels of pancreatic exocrine enzymes such as lipase and amylase, which is consistent with acute pancreatitis.

According to a nationwide survey in Japan, there were 15 cases of FT1D associated with DIHS/DRESS between 1985 and 2010. The mean age at onset of FT1D associated with DIHS/DRESS was 53.4 years; the interval between the onset of DIHS/DRESS to the development of FT1D was 39.9 days (range, 13–199 days). The incidence of FT1D in patients with DIHS/DRESS (0.54%) is much higher than that in the general Japanese population (0.010%).[53] The clinical manifestations of FT1D associated with DIHS/DRESS are similar to those not associated with DIHS/DRESS. It is possible that genetic susceptibility contributes to the development of FT1D. Notably, the incidence of HLA-B62 is significantly increased in this type of diabetes mellitus in Japanese patients with DIHS/DRESS. Viral reactivation may contribute to the development of FT1D in patients with DIHS/DRESS, based on the observation that FT1D is associated with viral infections such as influenza B, HHV-6, herpes simplex virus, and Coxsackie B3 virus.[54,55] In this setting, the rapid and severe damage to pancreatic β-cells may be caused by viral infections, an immune response, or an interplay between viruses and the immune response.[56]

By contrast, autoimmune T1D is rare in patients with DIHS/DRESS. In autoimmune T1D, various autoantibodies including anti-glutamic acid decarboxylase (GAD) and islet cell antibodies are detected. The coexistence of autoimmune T1D and autoimmune thyroiditis has been associated with DIHS/DRESS.[45] In this case, various autoantibodies including insulinoma antigen 2 (IA2), anti-GAD, anti-TPO, anti-thyroglobulin, and anti-SSA antibodies and ANA were detected over a period of several months. A case of T1D following methimazole-induced hypersensitivity syndrome has been reported,[51] in which high glucose levels with a low serum C-peptide were detected 5 months after the onset of DIHS/DRESS in a patient with Graves' disease. Interestingly, anti-GAD antibodies were detected but at a relatively low level. A case of FT1D and Hashimoto's disease that developed concurrently after the onset of DIHS/DRESS has been reported, characterized by the presence of anti-thyroglobulin antibodies, ANA, and anti-SSA antibodies with an absence of GAD and islet cell antibodies.[57]

The consequences of missing the diagnosis of T1D can be fatal. It is essential to recognize the initial symptoms of T1D in patients with DIHS/DRESS in order to initiate appropriate treatment.

Other sequelae in DIHS/DRESS

Besides autoimmune thyroiditis and T1D, other autoimmune sequelae—heralded by autoimmune manifestations and/or presence of autoantibodies—can arise after resolution of DIHS/DRESS following a symptom-free interval of several months to years. These autoimmune diseases include sclerodermoid graft-versus-host disease (GVHD)-like lesions,[40] lupus erythematosus,[58] autoimmune hemolytic anemia (AIHA),[2] and rheumatoid arthritis (Table 2).

The authors have encountered three interesting cases of DIHS/DRESS with autoimmune sequelae of sclerodermoid GVHD-like lesions and autoimmune thyroiditis, atypical SLE, and rheumatoid arthritis, which appeared 3.5 years, 4 years, and 10 years after resolution of DIHS/DRESS, respectively. The first patient had a history of zonisamide-induced DIHS/DRESS and presented with fatigue and symptoms of thyroid dysfunction; diffuse alopecia on the scalp and multiple ill-defined brownish, indurated plaques with xerosis on the

Table 2 Autoimmune diseases as sequelae in DIHS/DRESS.

Alopecia
AIHA
Graves' disease
Hashimoto's disease
Lupus erythematosus
Rheumatoid arthritis
Sclerodermoid GVHD-like lesion
T1D (fulminant and autoimmune)

AIHA = autoimmune hemolytic anemia; DIHS/DRESS = drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms; GVHD = graft-versus-host disease; T1D = type 1 diabetes mellitus.

extremities were observed on examination.[40] Interestingly, ANA was negative during the course of DIHS/DRESS, but was detectable at the time of presentation to our hospital.[40] The second patient had a history of carbamazepine-induced DIHS/DRESS with reactivation of HHV-6 and EBV, and presented with a high-grade fever, general fatigue, cervical lymphadenopathy, and erythematous lesions on his face and ears.[58] After resolution of DIHS/DRESS, he developed prominent cervical lymphadenopathy. Histological findings of a lymph node biopsy specimen were compatible with those of Kikuchi-Fujimoto disease, and expression of EBV-encoded RNA (EBER) was detected in the lymph node by *in situ* hybridization, but not in the blood. Clinical manifestations of SLE including fever, general fatigue, discoid lesions, leucopenia, and proteinuria appeared 2 weeks after the onset of Kikuchi-Fujimoto disease. The third patient had a history of carbamazepine-induced DIHS/DRESS with the appearance of autoantibodies such as ANA, anti-TPO, and anti-thyroglobulin antibodies after the resolution of DIHS/DRESS. The autoantibody levels fluctuated without overt clinical symptoms. Ten years after the onset of DIHS/DRESS, the patient developed rheumatoid arthritis with characteristic joint deformities of the hands. It was noted that the second and third cases of DIHS/DRESS had been treated with supportive therapy alone. Chen et al described a case of AIHA and suspected SLE after the resolution of dapsone-induced DIHS/DRESS.[2] In that case, the patient had a high lactate dehydrogenase level, elevated percentage of reticulocytes, decreased haptoglobin concentration, and a positive Coombs test.

Pathomechanism of autoimmune disease in DIHS/DRESS

The pathomechanism underlying the emergence of autoimmune disease in DIHS/DRESS is poorly understood. A genetic susceptibility may contribute to their development.[53] Based on long-term follow-up of patients with DIHS/DRESS, several observations regarding the development of autoimmune sequelae were noted: young patients[2] and those treated with non-corticosteroid therapy were more susceptible,[3] and that herpesvirus reactivation—in particular, Epstein-Barr virus reactivation—is implicated in its development.[3,56]

From an immunological perspective, our previous study showed that the number of fully functional $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells is markedly increased during the course of DIHS/DRESS compared with other drug reactions, which contributed to viral reactivation. These $FoxP3^+$ T cells lost their ability to inhibit the cytokine production and proliferation of effector T cells, which coincided with their contraction upon clinical resolution of DIHS/DRESS. The functional defect of Treg cells might be responsible for the development of autoimmune disease.[8,56,59]

Conclusion

In summary, the development of long-term sequelae after resolution of severe cutaneous adverse drug reactions may be overlooked because of an asymptomatic interval after resolution of the acute disease. The emergence of sequelae should be closely monitored following the resolution of SJS/TEN and DIHS/DRESS, especially autoimmune disease in DIHS/DRESS. The reactivation of herpesviruses and development of autoimmune disease in DIHS/DRESS may indicate a possible link between viral infections and autoimmune disease.

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References

1. Sotozono C, Ang LP, Koizumi N, Higashihara H, Ueta M, Inatomi T. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. *Ophthalmology* 2007;**114**:1294–302.
2. Chen YC, Chang CY, Cho YT, Chiu HC, Chu CY. Long-term sequelae of drug reaction with eosinophilia and systemic symptoms: a retrospective cohort study from Taiwan. *J Am Acad Dermatol* 2013;**68**:459–65.
3. Ushigome Y, Kano Y, Ishida T, Hirahara K, Shiohara T. Short- and long term outcomes of 34 patients with drug-induced hypersensitivity syndrome in a single institution. *J Am Acad Dermatol* 2013;**68**:721–8.
4. Valeyrie-Allanore L, Bastuji-Garin S, Guégan S, et al. Prognostic value of histologic features of toxic epidermal necrolysis. *J Am Acad Dermatol* 2013;**68**: e29–35.
5. Sekula P, Dunant A, Mockenhaupt M, et al. Comprehensive survival analysis of a cohort of patients with Stevens-Johnson syndrome and toxic epidermal necrolysis. *J Invest Dermatol* 2013;**133**:1197–204.
6. Tohyama M, Watanabe H, Murakami S, et al. Possible involvement of CD14+ CD16+ monocyte lineage cells in the epidermal damage of Stevens-Johnson syndrome and toxic epidermal necrolysis. *Br J Dermatol* 2012;**166**:322–30.
7. Azukizawa H, Sano S, Kosaka H, Sumikawa Y, Itami S. Prevention of toxic epidermal necrolysis by regulatory T cells. *Eur J Immunol* 2005;**35**:1722–30.
8. Takahashi R, Kano Y, Yamazaki Y, Kimishima M, Mizukawa Y, Shiohara T. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. *J Immunol* 2009;**182**:8071–9.
9. Viard I, Wehrli P, Bullani R, et al. Inhibition of toxic epidermal necrolysis by blocade of CD95 with human intravenous immunoglobulin. *Science* 1998;**282**: 490–3.
10. Nassif A, Moslehi H, Le Gouvello S, et al. Evaluation of the potential role of cytokines in toxic epidermal necrolysis. *J Invest Dermatol* 2004;**123**:850–5.
11. Nomura Y, Aihara M, Matsukura S, et al. Evaluation of serum cytokine levels in toxic epidermal necrolysis and Stevens-Johnson syndrome compared with other delayed-type adverse drug reactions. *J Dermatol* 2011;**38**:1076–9.
12. Chung WH, Hung SI, Yang JY, et al. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008;**14**:1343–50.
13. Lin A, Patel N, Yoo D, DeMartelaere S, Bouchard C. Management of ocular conditions in the burn unit: thermal and chemical burns and Stevens-Johnson syndrome/toxic epidermal necrolysis. *J Burn Care Res* 2011;**32**:547–60.
14. Kunimi Y, Hirata Y, Aihara M, Yamane Y, Ikezawa Z. Statistical analysis of Stevens-Johnson syndrome caused by mycoplasma pneumonia infection in Japan. *Allergol Int* 2011;**60**:525–32.
15. Meyer Sauteur PM, Goetschel P, Lautenschlager S. *Mycoplasma pneumoniae* and mucositis – part of the Stevens-Johnson syndrome spectrum. *J Dtsch Dermatol Ges* 2012;**10**:740–6.
16. Ueta M, Tokunaga K, Sotozono C, et al. HLA-A* 0206 with TLR3 polymorphisms exerts more than additive effects in Stevens-Johnson syndrome with severe ocular surface complications. *PLoS One* 2012;**7**:e43650.
17. Kaser DJ, Reichman DE, Laufer MR. Prevention of vulvovaginal sequelae in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Rev Obstet Gynecol* 2011;**4**:81–5.
18. De Jesus LE, Dekermacher S, Manhães CR, Faria LM, Barros ML. Acquired labial sinechiae and hydrocolpos secondary to Stevens-Johnson syndrome. *Urology* 2012;**80**:919–21.
19. Sibaud V, Fricain JC, Lèautè-Labrèze C, Campana F, Taieb A. Persistant mucosal ulcerations: a rare complication of toxic epidermal necrolysis. *Ann Dermatol Venereol* 2005;**132**:682–5.
20. Kreft B, Lieser U, Haase R, Marsch WC, Wohlrab J. Extensive hypertrophic scarring after toxic epidermal necrolysis in a child. *Pediatr Dermatol* 2012. http://dx.doi.org/10.1111/j.1525-1470.2012.01863.x.

21. Sotozono C, Ueta M, Koizumi N, et al. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. *Ophthalmology* 2009;**116**:685–90.
22. Roujeau JC, Phlippoteau C, Koso M, Wechsler J, Andre C, Binaghi M. Sjögren-like syndrome after drug induced toxic epidermal necrolysis. *Lancet* 1985;**1**:609–11.
23. Akosa AB, Elhag AM. Toxic epidermal necrolysis a study of the sweat glands. *J Cutan Pathol* 1995;**22**:359–64.
24. Shoji T, Cockerell CL, Koff AB, Bhawan J. Eruptive melanocytic nevi after Stevens-Johnson syndrome. *J Am Acad Dermatol* 1997;**37**:337–9.
25. Moosbrugger EA, Adams BB. Disseminated eruptions of ectopic sebaceous glands following Stevens-Johnson syndrome. *J Am Acad Dermatol* 2011;**65**:446–8.
26. Woo T, Saito H, Yamakawa Y, Komatsu S, Onuma S, Okudela K. Severe obliterative bronchitis associated with Stevens-Johnson syndrome. *Intern Med* 2011;**50**:2823–7.
27. Kamada N, Kinoshita K, Togawa Y, Kobayashi T, Matsubara H, Kohno M. Chronic pulmonary complications associated with toxic epidermal necrolysis: report of a severe case with anti-Ro/SS-A and a review of the published work. *J Dermatol* 2006;**33**:616–22.
28. Tan YM, Goh K. Esophageal stricture as a late complication of Stevens-Johnson syndrome. *Gastrointest Endosc* 1999;**50**:566–8.
29. Misra SP, Dwivedi M, Misra V. Esophageal stricture as a late sequel of Stevens-Johnson syndrome in adults: incidental detection because of foreign body impaction. *Gastrointest Endosc* 2004;**59**:437–40.
30. Descamps V, Bouscarat F, Laglenne S, et al. Human herpesvirus 6 infection associated with anticonvulsant hypersensitivity syndrome and reactive haemophagocytic syndrome. *Br J Dermatol* 1997;**137**:605–8.
31. Suzuki Y, Inagi R, Aono T, Yamanishi K, Shiohara T. Human herpesvirus 6 infection as a risk factor for the development of severe drug-induced hypersensitivity syndrome. *Arch Dermatol* 1998;**134**:1108–12.
32. Tohyama M, Yahata Y, Yasukawa M, et al. Severe hypersensitivity syndrome due to sulfasalazine associated with reactivation of human herpesvirus 6. *Arch Dermatol* 1998;**134**:1113–7.
33. Hashimoto K, Yasukawa M, Tohyama M. Human herpesvirus 6 and drug allergy. *Curr Opin Allergy Clin Immunol* 2003;**3**:255–60.
34. Chiou CC, Yang LC, Hung SI, et al. Clinicopathological features and prognosis of drug rash with eosinophilia and systemic symptoms: a study of 30 cases in Taiwan. *J Eur Acad Dermatol Venereol* 2008;**22**:1044–9.
35. Kano Y, Ishida T, Hirahara K, Shiohara T. Visceral involvements and long-term sequelae in drug-induced hypersensitivity syndrome. *Med Clin North Am* 2010;**94**:743–59.
36. Chen YC, Chiu HC, Chu CY. Drug reaction with eosinophilia and systemic symptoms. A retrospective study of 60 cases. *Arch Dermatol* 2010;**146**:1373–9.
37. Wei CH, Chung-Yee Hui R, Chang CJ, et al. Identifying prognostic factors for drug rash with eosinophilia and systemic symptoms (DRESS). *Eur J Dermatol* 2011;**21**:930–7.
38. Cacoub P, Musette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med* 2011;**124**:588–97.
39. Wongkitisophon P, Chanprapaph K, Rattanakaemakorn P, Vachiramon V. Six-year retrospective review of drug reaction with eosinophilia and systemic symptoms. *Acta Derm Venereol* 2012;**92**:200–5.
40. Kano Y, Sakuma K, Shiohara T. Sclerodermoid graft-versus-host disease-like lesions occurring after drug-induced hypersensitivity syndrome. *Br J Dermatol* 2007;**156**:1061–3.
41. Shiohara T, Kano Y, Takahashi R, Ishida T, Mizukawa Y. Drug-induced hypersensitivity syndrome: recent advances in the diagnosis, pathogenesis and management. *Chem Immunol Allergy* 2012;**97**:122–38.
42. Ishida T, Inaoka M, Hirahara K, Kano Y, Shiohara T. Enhanced production of autoantibodies following resolution of drug-induced hypersensitivity syndrome. *Rinsyo Hifuka* 2010;**64**:260–4 in Japanese.
43. Cookson H, Creamer D, Walsh S. Thyroid dysfunction in drug reaction with eosinophilia and systemic symptoms (DRESS): an unusual manifestation of systemic drug hypersensitivity. *Br J Dermatol* 2013;**168**:1130–2.
44. Teo RY, Tay YK, Tan CH, Ng V, Oh DC. Presumed dapsone-induced drug hypersensitivity syndrome causing reversible hypersensitivity myocarditis and thyrotoxicosis. *Ann Acad Med Singap* 2006;**35**:833–6.
45. Brown RJ, Rother KI, Artman H, Mercurio MG, Wang R, Looney RJ. Minocycline-induced drug hypersensitivity syndrome followed by multiple autoimmune sequelae. *Arch Dermatol* 2009;**145**:63–6.
46. Funck-Brentano E, Duong T, Family D, et al. Auto-immune thyroiditis and drug reaction with eosinophilia and systemic symptoms (DRESS) associated with HHV-6 viral reactivation. *Ann Dermatol Venereol* 2011;**138**:580–5.
47. Descamps V. DRESS (drug reaction with eosinophilia and systemic symptoms) and thyroiditis: HHV-6, the possible common link. *Br J Dermatol* 2013. http://dx.doi.org/10.1111/bjd.12456.
48. Caselli E, Zatelli MC, Rizzo R, et al. Virological and immunological evidence supporting an association between HHV-6 and Hashimoto's thyroiditis. *PloS Pathog* 2012;**8**:e1002951.
49. Sekine N, Motokura T, Oki T, et al. Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. *JAMA* 2001;**285**:1153–4.
50. Sommers LM, Schoene RB. Allopurinol hypersensitivity syndrome associate with pancreatic exocrine abnormalities and new-onset diabetes mellitus. *Arch Intern Med* 2002;**162**:1190–2.
51. Ozaki N, Miura Y, Sakakibara A, Oiso Y. A case of hypersensitivity syndrome induced by methimazole for Graves' disease. *Thyroid* 2005;**15**:1333–6.
52. Chiou CC, Chung WH, Hung SI, Yang LC, Hong HS. Fulminant type 1 diabetes mellitus caused by drug hypersensitivity syndrome with human herpesvirus 6 infection. *J Am Acad Dermatol* 2006;**54**(Suppl. 2):S14–7.
53. Onuma H, Tohyama M, Imagawa A, et al. High frequency of HLA B62 in fulminant type 1 diabetes with the drug-induced hypersensitivity syndrome. *J Clin Endocrinol Metab* 2012;**97**:E2277–81.
54. Sano H, Terasaki J, Tsutsumi C, Imagawa A, Hanafusa T. A case of fulminant type 1 diabetes mellitus after influenza B infection. *Diabetes Res Clin Pract* 2008;**79**:e8–9.
55. Van der Welf N, Kroese FG, Rozing J, Hillebrands JL. Viral infections as potential triggers of type 1 diabetes. *Diabetes Metab Res Rev* 2007;**23**:169–83.
56. Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol* 2007;**33**:124–33.
57. Minegaki Y, Higashida Y, Ogawa M, Miyachi Y, Fujii H, Kabashima K. Drug-induced hypersensitivity syndrome complicated with concurrent fulminant type1diabetes mellitus and Hashimoto's thyroiditis. *Int J Dermatol* 2013;**52**:355–7.
58. Aota N, Hirahara K, Kano Y, Fukuoka T, Yamada A, Shiohara T. Systemic lupus erythematosus presenting with Kikuchi-Fujimoto's disease as a long-term sequela of drug-induced hypersensitivity syndrome, a possible role of Epstein-Barr virus reactivation. *Dermatology* 2009;**218**:275–7.
59. Aota N, Shiohara T. Viral connection between drug rashes and autoimmune diseases: how autoimmune responses are generated after resolution of drug rashes. *Autoimmun Rev* 2009;**8**:488–94.

LETTER

RESEARCH LETTER

Methylprednisolone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis: Clinical evaluation and analysis of biomarkers

To the Editor: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe, potentially life-threatening, cutaneous reactions.[1] Progress has been made in determining the pathophysiologic mechanisms of SJS/TEN; however, there has been little progress in the treatment of these conditions.[2,3] Thus, the efficacy of steroid pulse therapy and alterations of biomarkers were assessed retrospectively.

The medical records of 8 patients treated with methylprednisolone pulse therapy (MPT) for SJS/TEN were reviewed, documenting clinical findings and specific biomarkers. The diagnosis of SJS/TEN was based on Bastuji-Garin criteria.[4] High-grade fever was present on admission in 6 of 8 patients and all had mucosal lesions. Causative agents were identified in 7 of 8 patients. These agents were nonsteroidal anti-inflammatory drug (diclofenac and loxoprofen), antimicrobial agent (garenoxacin and penicillin), anticonvulsant (lamotrigine and zonisamide), and bromhexine. Notable coexisting conditions included malignancy (lung and colon) in two patients, cerebral hematoma complicated by tachycardia and diabetes mellitus in 1 patient, and increased serum creatinine in 1 patient. In the calculation of SCORTEN (a severity-of-illness score for TEN that predicts mortality),[5] the mean SCORTEN on admission was 2.1 (range, 0-4) (Table I). [T1]

An infusion of methylprednisolone at 1000 mg/d for 3 consecutive days was administered. Oral prednisolone at 0.8-1 mg/kg/d was initiated if no new mucocutaneous lesions were observed on the day following the last dose of MPT, and prednisolone was subsequently tapered every week. If the high-grade fever persisted or a reduction in body surface area (BSA) involvement was not observed after the last dose of MPT, a course of half-dose methylprednisolone pulse therapy (500 mg/d for 2 consecutive days) was administered.

No patients died during the follow-up period of 3 months, whereas predicted mortality was 1.6 deaths according to SCORTEN. In 7 of 8 patients after initiation of MPT, the mean BSA of epidermal detachment was reduced at day 4, and complete re-epithelialization was observed in a mean interval of 12.7 ± 7.5 days (range, 7-28 days). Plasma exchange was given to 1 patient in whom the BSA had increased from 70% to 80% and was accompanied by a high-grade fever. Severe bacterial infection, such as sepsis, pneumonia, and pyelitis, was not observed during or after MPT. Cytomegalovirus antigenemia was detected in 2 patients at 10 and 25 days after the last dose of MPT. A 7-day course of ganciclovir was given to both patients. Increase in hepatitis B virus load was detected in 1 patient who was an asymptomatic carrier.

Table I. Patient characteristics and clinical evaluation

No. Age, y / Sex	Diagnosis	Previous treatment (day)	Interval day*	SCOR-TEN†	Treatment	BSA of detachment lesion (%)			Re-epitheliali-zation day§
						Day 0	Day 4	Day 7 or 8	
1/31/F	SJS	PSL 50 mg/d (3)	10	0	MPT	5	4	2	13
2/66/M	SJS	Hydrocortisone 100 mg/d (1)	5	2	MPT	8	6	1	8
3/74/M	SJS	IVIg 20 g/d (3)	3	4	MPT + HD-MPT	8	6	5	28
4/32/F	Overlap	PSL 30 mg/d (4)	6	2	MPT	15	12	1	8
5/40/F	Overlap	PSL 60 mg/d (3)	8	2	MPT	15	10	0	7
6/48/M	TEN	PSL 20 mg/d (4)	5	2	MPT	80	72	10	9
7/48/F	TEN	PSL 60 mg/d (3)	6	2	MPT + HD-MPT	35	20	5	16
8/70/F	TEN	PSL 60 mg/d (2)	3	3	MPT➡PE	70	80	NA	NA

BSA, Body surface area; *HD-MPT,* half-dose MPT; *IVIg,* intravenous immunoglobulin; *MPT,* methylprednisolone pulse therapy; *NA,* not applicable; *Overlap,* overlap of Stevens-Johnson syndrome and toxic epidermal necrolysis; *PE,* plasma exchange; *PLS,* prednisolone; *SCORTEN,* severity-of-illness score for toxic epidermal necrolysis; *SJS,* Stevens-Johnson syndrome; *TEN,* toxic epidermal necrolysis; *y,* year; ➡, switched to.

*Between onset of disease and initiation of MPT.

†Level of serum bicarbonate was not measured.

§Interval between initiation of MPT and complete re-epithelialization.

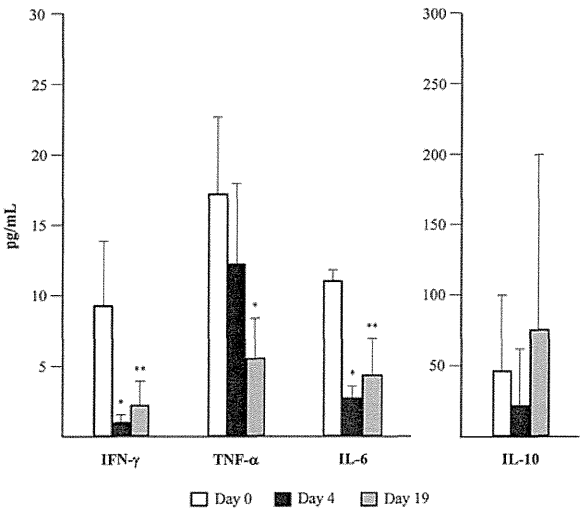

Fig 1. Alterations in cytokine levels. *Day 0,* Before methylprednisolone pulse therapy (MPT); *Day 4,* the day after the third dose of MPT or last administration of half-dose MPT, *Day 19,* mean 19 days (range, 13-28 days) from day 0; *IFN,* Interferon; *IL,* interleukin; *TNF,* tumor necrosis factor. Cytokine levels were measured using bead-based multiplexing assays (Luminex xMAP® technology). $^{*}P < .05$, $^{**}P < .005$; compared with day 0.

Biomarkers (serum interferon [IFN]-γ, tumor necrosis factor [TNF]-α, interleukin [IL]-6 and IL-10) were available for 5 of 8 patients. At day 4 after MPT administration, mean levels of IFN-γ, TNF-α, IL-6, and IL-10 were decreased compared with preadministration levels (day 0), but only changes in IFN-γ and IL-6 reached statistical significance. At day 19, a significant reduction in the mean levels of IFN-γ, TNF-α, and IL-6 was observed compared with levels before administration-of MPT, while the mean level of IL-10 was higher than that on day 0, although the difference was not statistically [F1] significant (Fig 1).

The decrease in proinflammatory cytokine levels suggests that MPT may have contributed to the survival of these patients with drug-induced SJS/TEN. Large-scale, prospective, blinded trials will be required to prove that MPT is effective in the treatment of SJS/TEN.

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REFERENCES

1. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drug. N Engl J Med 1994;331:1272-85.
2. Valeyrie-Allanore L, Wolkenstein P, Brochard L, Ortonne N, Maître B, Revuz J, et al. Open trial of ciclosporin treatment for Stevens-Johnson syndrome and toxic epidermal necrolysis. Br J Dermatol 2010;163:847-53.
3. Kardaun SH, Jonkman MF. Dexamethasone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis. Acta Derm Venereol 2007;87:144-8.
4. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. Arch Dermatol 1993;129:92-6.
5. Bastuji-Garin S, Fouchard N, Bertocchi M, Roujeau JC, Revuz J, Wolkenstein P. SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol 2000;115:149-53.

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Short- and long-term outcomes of 34 patients with drug-induced hypersensitivity syndrome in a single institution

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Background: Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe systemic hypersensitivity reaction caused by specific drugs, in which herpesvirus reactivations and organ dysfunction occur during the course of the disease. Although recent reports have documented the development of autoimmune disease after complete resolution of DIHS/DRESS, relatively little is known about long-term outcomes after complete resolution of the disease.

Objective: The aim of this study was to retrospectively analyze complications and sequelae in the early and late phases of DIHS/DRESS according to treatment.

Methods: In all, 34 patients were classified into 2 groups: 14 patients with oral corticosteroid treatment; and 20 with noncorticosteroid treatment. The disease time course was divided into 2 periods: the first 6 months after onset of the drug reaction (early phase); and the period thereafter (late phase). Investigations to detect the presence of viral/bacterial infectious diseases, organ dysfunction, and autoantibodies were performed in both early and late phases.

Results: Herpesvirus infections and pneumonia were detected in 6 and 2 patients, respectively, in the corticosteroid treatment group in the early phase. In the noncorticosteroid treatment group, 2 patients developed autoimmune diseases, namely lupus erythematosus and autoimmune thyroiditis. Autoantibodies were detected in 44.4% of patients examined in the late phase of the disease.

Limitations: This study only evaluated a small number of autoantibodies.

Conclusion: The need for anti-inflammatory effects from systemic corticosteroids should be balanced with the risk of infectious diseases and the benefits of preventing the appearance of later autoimmune conditions in patients with DIHS/DRESS. (J Am Acad Dermatol 2013;68:721-8.)

Key words: complication; corticosteroid; drug-induced hypersensitivity syndrome; drug reaction; drug reaction with eosinophilia and systemic symptoms; herpesvirus; outcome; treatment; viral reactivation.

Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe systemic hypersensitivity reaction caused by specific drugs such as anticonvulsants and allopurinol, and is characterized by organ dysfunction and reactivation of human herpesvirus (HHV)-6.[1-3] Reactivation of other herpesvirus, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) may occur during the course of this drug reaction.[4-6] Despite the complete recovery from DIHS/DRESS, the development of autoimmune sequelae such as autoimmune

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thyroiditis, sclerodermoid lesions, type 1 diabetes mellitus, and lupus erythematosus has been reported.[7-12] The long-term outcomes of DIHS/DRESS after complete resolution of the disease are unclear, because of a lack of long-term follow-up and the potential development of sequelae after a disease-free period of several months to years. In particular, the relationship between administration of systemic corticosteroids—a common treatment for DIHS/DRESS—and long-term outcome is not well documented.[13,14] Long-term outcomes may be influenced by the type and duration of treatment, herpesvirus reactivation, genetic factors, and the presence of underlying disease. To clarify the relationship between treatment and outcome in DIHS/DRESS, we retrospectively analyzed the complications and sequelae in the early and late phases of the disease in relation to treatments in patients with DIHS/DRESS seen at our institution. This study revealed significant differences in outcomes according to treatment for DIHS/DRESS.

CAPSULE SUMMARY

- Drug-induced hypersensitivity syndrome is a severe systemic hypersensitivity reaction, and involves the reactivation of herpesviruses. Various infections and organ failure can develop during the course of this disease.
- Development of autoimmune diseases and autoantibodies were detected in noncorticosteroid-treated patients after complete resolution of the disease.
- Treatments for drug-induced hypersensitivity syndrome should be carefully selected based on an understanding of the differences in treatment modalities.

METHODS

Patients

This study was approved by the institutional review board of Kyorin University School of Medicine, Tokyo, Japan. The medical records of 40 patients who had been admitted into our hospital for DIHS/DRESS between 1998 and 2010 were reviewed. All patients satisfied the diagnostic criteria for DIHS/DRESS proposed by the Japanese Severe Cutaneous Adverse Reaction Group,[15] and the culprit drug had been discontinued once the diagnosis was suspected. Patients were excluded from further analysis if: they were older than 85 years; significant underlying diseases were present, including heart or renal failure; previous treatment before visiting our hospital was unclear; or if the period of observation and follow-up was less than 1 year after the initiation of treatment in our hospital. After the exclusion of ineligible patients based on the exclusion criteria, 34 of the 40 patients given the diagnosis of DIHS/DRESS were enrolled in the study. Using the RegiSCAR scoring system proposed by Kardaun et al,[16] the 34 cases were classified as either definite or probable.

Patients were classified into 2 groups according to whether they had been treated with oral corticosteroids (corticosteroids, n = 14; noncorticosteroid treatment, n = 20). No other immunosuppressive agents had been administered. The initial oral corticosteroid dose was 0.6 to 1.0 mg/kg daily, after which the dose was gradually tapered. Most patients required more than 8 weeks of oral corticosteroids to achieve complete resolution. Patients who had received less than 0.25 mg/kg daily within 3 days before hospital admission were not included in the oral corticosteroid treatment group. A total of 5 patients in the corticosteroid treatment group had also received intravenous immunoglobulin (IVIG) therapy, with a dose of 5 g daily administered for 3 to 5 days on detection of herpesvirus reactivation. In the noncorticosteroid treatment group, 3 patients had received IVIG therapy with intravenous fluids. Two patients were given doses of 5 g daily for 3 and 5 days, respectively, and 1 patient who had 1 kidney because of previous excision of a renal tumor was given a dose of 2.5 g daily for 3 days. The other 17 patients were given supportive treatment with intravenous fluids (Fig 1). Some patients received topical corticosteroids for symptomatic relief. The type of treatment selected was based on the clinical judgment of the consulting dermatologist rather than a predetermined treatment algorithm.

The clinical features and culprit drugs in each group are shown in Table I. The respective mean age was 54.5 ± 19.7 and 56.4 ± 15.2 years in the corticosteroid and noncorticosteroid treatment groups. The type of culprit drugs and the presence of underlying disease were not significantly different between the 2 groups. In most patients, eruptions started as erythematous macules that enlarged and became confluent erythematous lesions. Mucosal lesions were present only in 1 patient in the corticosteroid treatment group. Skin biopsy specimens had been obtained from all patients and histopathological examination revealed scattered exocytosis of mononuclear cells in the epidermis and perivascular lymphocytic and eosinophilic infiltration in the papillary dermis in many specimens. Laboratory data including leukocyte and eosinophil counts,

Abbreviations used:

ATGA:	antithyroglobulin antibody
ATPOA:	antithyroperoxidase antibody
CMV:	cytomegalovirus
DIHS:	drug-induced hypersensitivity syndrome
DRESS:	drug reaction with eosinophilia and systemic symptoms
EBV:	Epstein-Barr virus
HHV:	human herpesvirus
IVIG:	intravenous immunoglobulin

C-reactive protein, alanine aminotransferase, and serum IgG levels in peripheral blood were obtained before treatment and analyzed to exclude differences in disease severity between the 2 groups; no significant differences were observed in any of these parameters between the 2 groups. The culprit drug was confirmed using the lymphocyte transformation test. Positive lymphocyte transformation test results were obtained more than 1 month after the onset of the drug reaction in most patients.

Assessment of clinical courses

The disease time course was divided into 2 periods: the first 6 months after the onset of a drug reaction was regarded as the early phase and the period thereafter was regarded as the late phase. The average time period of clinical observation (from disease onset until end of follow-up) was 53 and 41 months in the oral corticosteroid and noncorticosteroid treatment groups, respectively. Investigations to detect the presence of viral/bacterial infections and organ dysfunction attributable to DIHS/DRESS were performed in both the early and late phases. Mild liver dysfunction and/or erythematous rash commonly observed during the course of the disease were not considered to be complications of DIHS/DRESS.

Detection of viral reactivation

To detect HHV-6 reactivation, patients with suspected DIHS/DRESS were tested for anti-HHV-6 IgG antibody titers by fluorescent antibody assays and/or real-time polymerase chain reaction assays for HHV-6 DNA loads in peripheral leukocytes, based on *Taq*Man technology. HHV-6 reactivation was defined as a greater than 4-fold increase in anti-HHV-6 IgG antibody titers or detection of HHV-6 DNA in leukocytes. In addition, EBV and CMV DNA loads in peripheral leukocytes were also determined by means of polymerase chain reaction assays, based on *Taq*Man technology, during the course of the disease. DNA loads for herpesvirus were evaluated at either biweekly or triweekly intervals.

Detection of antibody

The presence of autoantibodies and increases in autoantibody levels were also evaluated in both phases. In some patients, serum was obtained before treatment and preserved at −80°C for measurement of autoantibodies including antinuclear antibody, antithyroglobulin antibody (ATGA), and antithyroperoxidase antibody (ATPOA), using a fluorescein-labeled antibody for determination of antinuclear antibody levels and radioimmunoassay for measurement of ATGA and ATPOA levels. The aforementioned antibodies were selected based on preliminary results that had shown no alterations in the levels of rheumatoid factor, antitopoisomerase 1, or antimitochondrial or antithyroglobulin receptor antibodies in patients' sera. Autoantibody levels were measured at intervals of several months in the majority of patients. Autoantibody levels were compared with those before the initiation of treatment.

Statistical analyses

Laboratory data from the 2 treatment groups were analyzed using Student *t* test. Values of *P* less than .05 were taken to indicate statistical significance.

RESULTS

The overall mortality was 8.8%. In the corticosteroid treatment group, various infections such as herpes labialis, herpes zoster, CMV diseases, and pneumonia were seen in the early phase. Herpes labialis was detected within 10 days after the onset of DIHS/DRESS. Herpes zoster occurred in 2 patients approximately 2 months after the onset of drug eruptions during corticosteroid tapering, and 1 month after the cessation of corticosteroid therapy in 1 patient. The cutaneous manifestations of herpes zoster were mild and resolved without any complications.[17] CMV diseases were noted in 1 patient, which manifested as gastrointestinal bleeding and skin ulcers on the back 5 weeks after the onset of DIHS/DRESS,[18] followed by acute respiratory distress syndrome, resulting in death. Limbic encephalitis, possibly associated with HHV-6 reactivation, occurred 3 weeks after the onset of DIHS/DRESS in 1 patient, and was complicated by syndrome of inappropriate secretion of antidiuretic hormone.[19] Some of the cases mentioned in the current study have already been published.[7,8,17-19] Most viral infections, including herpes zoster, encephalitis, and gastrointestinal bleeding, were detected when the dose of oral corticosteroid was decreased to 25% to 67% of the initial dose. In addition, 2 cases of pneumonia occurred: 1 patient died of acute respiratory distress syndrome secondary to *Pneumocystis jiroveci* pneumonia 2.5 months after the onset of

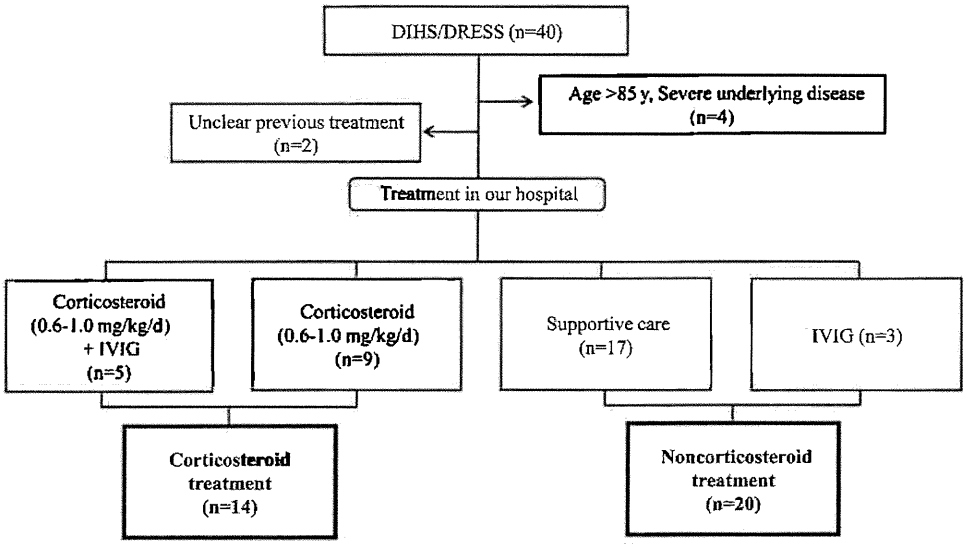

Fig 1. Patient flow diagram. Patients with drug-induced hypersensitivity syndrome (*DIHS*)/drug reaction with eosinophilia and systemic symptoms (*DRESS*) were divided into 2 groups. Administration of intravenous immunoglobulin (*IVIG*) was included in each group.

Table I. Patient characteristics

Treatment group (No. of cases)	Age, y, mean ± SD	M:F	Underlying disease (No. of cases)	Culprit drug (No. of cases)
Corticosteroid (14)	54.5 ± 19.7	10:4	Arrhythmia (1), cerebral infarction (2), colitis (1), convulsion (1), epilepsy (3), hyperuricemia (1), neuralgia (1), psychiatric disease (2), rheumatoid arthritis (1), vasculitis (1)	Allopurinol (1), carbamazepine (6), dapsone (1), mexiletine (1), phenobarbital (2), phenytoin (1), salazosulfapyridine (2)
Noncorticosteroid (20)	56.4 ± 15.2	8:12	Cerebral infarction (3), convulsion (5), encephalitis/asthma (1), epilepsy (1), hyperuricemia (1), hyperuricemia/Sjögren syndrome (1), hyperuricemia/hepatitis C/renal tumor (1), neuralgia (4), psychiatric disease (3)	Allopurinol (3), carbamazepine (14), phenobarbital (1), phenytoin (2)

F, Female; *M*, male.

DIHS/DRESS; and another patient with interstitial pneumonia developed *Cryptococcus* pneumonia 8 months after the onset of DIHS/DRESS. In these 2 patients, infectious pneumonia was observed when the doses of corticosteroid were reduced to 50% and 15% of the initial dose, respectively. Bacterial intramuscular abscess occurred in 1 patient when the dose was decreased to 75% of the initial dose (Table II).

In the early phase of DIHS/DRESS in the noncorticosteroid treatment group, diffuse alopecia developed 4 months after the onset of DIHS/DRESS in 1 patient without evidence of thyroid dysfunction, which persisted for 6 months. Another patient with gastrointestinal bleeding caused by CMV infection required emergency endoscopic clipping and administration of ganciclovir with IVIG.[18] In contrast to the corticosteroid treatment group, no bacterial infections were seen in the noncorticosteroid treatment group in the early phase (Fig 2).

Liver dysfunction was observed in all patients in the early phase. Severe liver dysfunction (alanine aminotransferase >300 IU/L) was detected in 8 patients, and in 4 of these 8 patients the corticosteroid was administered at the initial dose of 0.8 mg/kg daily. The others were managed with supportive treatment monitored by specialists. Liver dysfunction ameliorated within 80 days in most patients, with some fluctuations in the levels. No fatal sequelae were noted in the current study.

Table II. Onset of complications and corticosteroid treatment doses

Case No.	Age, y/gender	Underlying disease	Culprit drug	Complication after treatment	Corticosteroid (prednisolone) Initial dose, mg/d	Corticosteroid (prednisolone) Dose at onset of complication, mg/d
1	39/M	Psychiatric disease	Carbamazepine	HZ	40	10
2	63/M	Convulsion	Carbamazepine	HZ	40	20
3	70/F	Cerebral infarction	Phenytoin	HZ	40	0
4	69/M	Epilepsy	Phenobarbital	En	60	40
5	74/M	Arrhythmia	Mexiletine	GB → ARDS	50	25 → 20
6	79/M	Neuralgia	Carbamazepine	AC → PP → ARDS	50	40 → 25 → 25
7	28/F	Epilepsy	Carbamazepine	IA	80	60
8	68/M	Rheumatoid arthritis	Salazosulfapyridine	IP → CP	40	15 → 6*

AC, Acute cholangitis; *ARDS*, acute respiratory distress syndrome; *CP*, *Cryptococcus* pneumonia; *En*, encephalitis; *F*, female; *GB*, gastrointestinal bleeding; *HZ*, herpes zoster; *IA*, intramuscular abscess; *IP*, interstitial pneumonia; *M*, male; *PP*, *Pneumocystis jiroveci* pneumonia.
*Administration of corticosteroids continued because of rheumatoid arthritis.

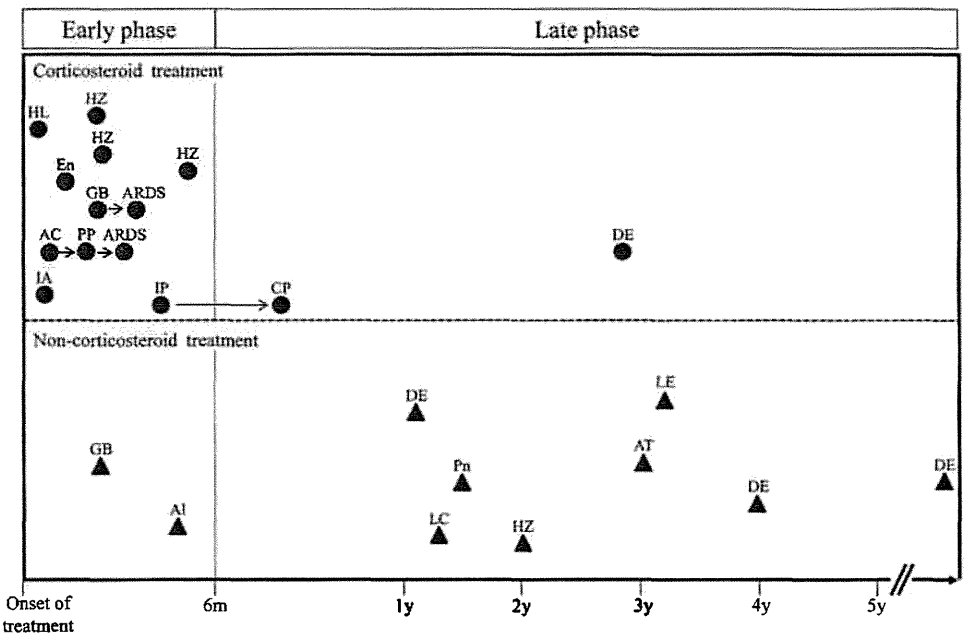

Fig 2. Complications and sequelae were classified into 2 groups in early and late phases according to treatment. *AC*, Acute cholangitis; *Al*, alopecia; *ARDS*, acute respiratory distress syndrome; *AT*, autoimmune thyroiditis; *CP*, *Cryptococcus* pneumonia; *DE*, drug eruption; *En*, encephalitis; *GB*, gastrointestinal bleeding; *HL*, herpes labialis; *HZ*, herpes zoster; *IA*, intramuscular abscess; *IP*, interstitial pneumonia; *LC*, lung cancer; *LE*, lupus erythematosus; *Pn*, pneumonia; *PP*, *Pneumocystis jiroveci* pneumonia.

In the late phase of DIHS/DRESS in the noncorticosteroid treatment group, autoimmune diseases developed in some patients. Lupus erythematosus with severe lupus nephritis developed in a patient 4 years after the onset of DIHS/DRESS treated with IVIG alone.[8] A case of asymptomatic autoimmune thyroiditis (Hashimoto thyroiditis) developed in a patient 3 years after the onset of DIHS/DRESS managed with supportive treatment alone. In this patient, thyroid stimulation hormone level increased 10 months after the onset of DIHS/DRESS, followed by detection of ATGA and ATPOA. One patient with a normal x-ray result on admission died of lung cancer 2 years after the onset of DIHS/DRESS. Drug eruptions were seen in 3 patients, caused by an antibiotic, an antilipemic agent, and a cold remedy, respectively (Fig 2). The lymphocyte transformation test levels were positive in 2 of these 3 cases.

Autoantibodies were more commonly detected in patients in the noncorticosteroid treatment group, particularly in the late phase, with 44.4% of patients

Table III. Detection of autoantibodies

	Detection of autoantibody	
	Frequency Detected antibodies (No. of cases)	
Treatment group (No. of cases)	Early phase	Late phase
Corticosteroid (6)	0% (0/6)	16.7% (1/6) ANA (1)
Noncorticosteroid (10)	20.0% (2/10) ANA (2)	70.0% (7/10) ANA (2) ATGA (4) ATPOA (3)

ANA, Antinuclear antibody; *ATGA*, antithyroglobulin antibody; *ATPOA*, antithyroperoxidase antibody.

Table IV. Herpesvirus reactivations

	Viral reactivation, no. of cases			
Treatment group (No. of cases)	HHV-6, EBV, CMV	HHV-6, EBV	HHV-6, CMV	HHV-6
Corticosteroid (13)	3	2	4	4
Noncorticosteroid (16)	2	7	1	6

CMV, Cytomegalovirus; *EBV*, Epstein-Barr virus; *HHV*, human herpesvirus.

demonstrating autoantibodies. One patient with positive ATPOA in the corticosteroid treatment group had a history of rheumatoid arthritis. Autoantibodies were present in all 3 patients who had been treated with IVIG alone (Table III). The respective ranges for antinuclear antibody, ATGA, and ATPOA detected were 40- to 320-fold, 1.5 to 8.1 U/L, and 1.0 to 19.5 U/L. These autoantibody titers remained elevated during the study period, with some fluctuations in the levels.

Herpesvirus reactivations detected during the course of the disease were classified into 4 groups: HHV-6, EBV, and CMV; HHV-6 and EBV; HHV-6 and CMV; and HHV-6 alone. Our results showed that CMV reactivation was detected in more than half of the patients in the corticosteroid treatment group. EBV reactivation was more frequently observed in patients in the noncorticosteroid treatment group than in the corticosteroid treatment group (Table IV).

DISCUSSION

Little attention has been paid to the long-term outcomes after complete recovery from DIHS/DRESS because many difficulties are encountered in following up with patients who do not seek medical care during a disease-free period after clinical resolution. This study revealed long-term outcomes obtained from a substantial number of patients with DIHS/DRESS who were carefully followed up by the same dermatologists in a single institution.

The prognosis of DIHS/DRESS may be influenced by age, genetic factors, presence of underlying disease, viral reactivation, and type of treatment. In particular, DIHS/DRESS appears to be worse in elderly patients, whereas younger patients recover more quickly. In the current study, there was no significant difference in age between the 2 groups, and laboratory data obtained before the initiation of therapy showed no significant differences between the 2 groups; thus, patient selection bias was unlikely.

Oral corticosteroids remain the mainstay treatment for DIHS/DRESS,[13,14] and a rapid resolution of symptoms is usually observed within several weeks after commencement. In this study, various infections were noted in the corticosteroid treatment group in the early phase, including herpesvirus diseases and *P jiroveci* pneumonia. CMV reactivation was more commonly detected in the corticosteroid treatment group, occurring in 53.8% examined. CMV disease and *P jiroveci* pneumonia were associated with delayed recovery and worse outcomes. Based on our results, most infectious diseases appeared within 3 months after initiation of the oral corticosteroid. As a result, careful follow-up for at least 3 months is recommended to minimize the risk of unfavorable outcomes in patients with DIHS/DRESS. The prolonged administration of oral corticosteroids may be partly responsible for these infections, given that the corticosteroid dose at 0.8 to 1.0 mg/kg daily was relatively high and administered over a long period, with the corticosteroid tapered over 2 months. However, considering the relatively high doses of corticosteroid used to treat other diseases such as collagen diseases and systemic vasculitis, it seems unlikely that corticosteroid alone was responsible for the infections in patients with DIHS/DRESS. In fact, 1 patient with gastrointestinal bleeding and skin ulcers caused by CMV disease had not been treated with oral corticosteroids.[18]

Alternatively, the development of infections in DIHS/DRESS during corticosteroid treatment may be analogous to the pathomechanisms of immune reconstitution inflammatory syndrome.[20] Diseases in the early stage of DIHS/DRESS such as herpes zoster, CMV infection, and *P jiroveci* pneumonia are similar to the range of illnesses in patients with AIDS and immune reconstitution inflammatory syndrome after highly active antiretroviral therapy.[21] As infectious diseases occurred most commonly during corticosteroid tapering down to 25%, the dose reduction of corticosteroids in the setting of DIHS/DRESS might have contributed to the appearance of these infectious diseases. An awareness of these infectious

diseases during tapering will facilitate prompt interventions in patients with DIHS/DRESS.

The development of autoimmune diseases such as lupus erythematosus[8] and autoimmune thyroiditis, along with the presence of autoantibodies, were observed in the noncorticosteroid treatment group in the late phase of DIHS/DRESS. Our previous article reported a patient who developed sclerodermoid graft-versus-host disease–like lesions treated with oral corticosteroids and low-dose IVIG in another hospital.[7] However, surprisingly, no cases of autoimmune disease were seen in the corticosteroid treatment group in the current study, and the appearance of autoantibodies was uncommon. It has been shown that IVIG compensates for the decreased immunoglobulin concentration, provides anti-inflammatory effects, and regulates the immune response in autoimmune diseases. However, according to Joly et al,[22] IVIG in patients with DIHS/DRESS can result in severe adverse events, which may require systemic corticosteroid therapy. In the current study, autoantibodies were detected in all 3 patients treated with IVIG. As only a small number of patients were enrolled in this study, it is difficult to determine the role of IVIG in the management of DIHS/DRESS. Further studies on treatment outcomes and long-term follow-up are thus needed in a larger patient population. Clearly, further work needs to be done regarding the link between the onset of DIHS/DRESS and the occurrence of cancer.

It is unclear why autoimmune diseases develop in the noncorticosteroid treatment group in DIHS/DRESS. We have already reported that regulatory T cells were expanded in the acute stage with normal functions, whereas regulatory T cells were functionally impaired in the resolution stage in patients with DIHS/DRESS.[23] These regulatory T cells most likely increased the susceptibility of patients with DIHS/DRESS to autoimmune diseases. However, autoimmune reactions could not be detected in all patients with DIHS/DRESS. Impaired regulatory T-cell function might thus contribute partially to the development of autoimmune diseases, but other pathomechanisms might also be responsible for the appearance of autoimmune reactions. On the other hand, we speculate that this may be linked to the EBV reactivation, because EBV shows a unique characteristic infection pattern of B cells. EBV infection might be a continuous source of chronic immune stimulation.[24] In fact, EBV has been implicated in the development of autoimmune diseases, such as lupus erythematosus and multiple sclerosis.[25,26] In the current study, EBV reactivation was more frequently detected in patients in the noncorticosteroid treatment group, with 56.3% of patients examined. This frequency was much higher than that in patients in the corticosteroid treatment group. In this regard, we have detected that viral loads of EBV are lower in the corticosteroid treatment group than in the noncorticosteroid treatment group, whereas viral loads of HHV-6 and CMV are higher in the corticosteroid treatment group than in the noncorticosteroid group (in preparation by Ishida). These findings may indicate that the pattern of viral reactivations enhanced by systemic corticosteroids would differ according to the virus.

Although only limited information could be obtained concerning the detection of autoantibodies, the frequency of the detection for autoimmune thyroid antibodies was markedly higher in the noncorticosteroid treatment group than in the corticosteroid treatment group. Considering that EBV antigens were found in target organs in multiple sclerosis,[27] the thyroid gland might be one of the target organs in this setting.

Our findings suggest that patients with DIHS/DRESS should be monitored for the development of autoimmune disease including laboratory examination of autoantibodies, despite clinical symptoms. The beneficial effects of oral corticosteroids in the suppression of inflammation and prevention of autoimmune disease need to be counterbalanced against the risk of infection.

REFERENCES

1. Descamps V, Bouscarat F, Laglenne S, Aslangul E, Veber B, Descamps D, et al. Human herpesvirus 6 infection associated with anticonvulsant hypersensitivity syndrome and reactive hemophagocytic syndrome. Br J Dermatol 1997;137:605-8.
2. Suzuki Y, Inagi R, Aono T, Yamanishi K, Shiohara T. Human herpesvirus 6 infection as a risk factor for the development of severe drug-induced hypersensitivity syndrome. Arch Dermatol 1998;134:1108-12.
3. Tohyama M, Yahata Y, Yasukawa M, Inagi R, Urano Y, Yamanishi K, et al. Severe hypersensitivity syndrome due to sulfasalazine associated with reactivation of human herpesvirus 6. Arch Dermatol 1998;134:1113-7.
4. Kano Y, Hirahara K, Sakuma K, Shiohara T. Several herpesviruses can reactivate in a severe drug-induced multiorgan reaction in the same sequential order as in graft-versus-host disease. Br J Dermatol 2006;155:301-6.
5. Seishima M, Yamanaka S, Fujisawa T, Tohyama M, Hashimoto K. Reactivation of human herpesvirus (HHV) family members other than HHV-6 in drug-induced hypersensitivity syndrome. Br J Dermatol 2006;115:344-9.
6. Picard D, Janela B, Descamps V, D'Incan M, Courville P, Jacquot S, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. Sci Transl Med 2010;2:45ra62.
7. Kano Y, Sakuma K, Shiohara T. Sclerodermoid graft-versus-host disease–like lesions occurring after drug-induced hypersensitivity syndrome. Br J Dermatol 2007;156:1061-3.
8. Aota N, Hirahara K, Kano Y, Fukuoka T, Shiohara T. Systemic lupus erythematosus presenting with Kikuchi-Fujimoto's disease as a long-term sequel of drug-induced hypersensitivity

syndrome; a possible role of Epstein-Barr virus reactivation. Dermatology 2009;218:275-7.
9. Brown RJ, Rother KI, Artman H, Mercurio MG, Wanq R, Looney RJ, et al. Minocycline-induced drug hypersensitivity syndrome followed by multiple autoimmune sequelae. Arch Dermatol 2009;145:63-6.
10. Eshki M, Allanore L, Musette P, Milipied B, Grange A, Guillaume JC, et al. Twelve-year analysis of severe cases of drug reaction with eosinophilia and systemic symptoms: a cause of unpredictable multiorgan failure. Arch Dermatol 2009;145:67-72.
11. Sekine N, Motokura T, Oki T, Umeda Y, Sasaki N, Hayashi M, et al. Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. JAMA 2001;285:1153-4.
12. Chiou CC, Chung WH, Hung SI, Yang LC, Hong HS. Fulminant type 1 diabetes mellitus caused by drug hypersensitivity syndrome with human herpesvirus 6 infection. J Am Acad Dermatol 2006;54(Suppl):S14-7.
13. Chiou CC, Yang LC, Hung SI, Chang YC, Kuo TT, Ho HC, et al. Clinicopathological features and prognosis of drug rash with eosinophilia and systemic symptoms: a study of 30 cases in Taiwan. J Eur Acad Dermatol Venereol 2008;22:1044-9.
14. Cacoub P, Musette P, Descamps V, Meyer O, Speirs C, Finzi L, et al. The DRESS syndrome: a literature review. Am J Med 2011;124:588-97.
15. Shiohara T, Iijima M, Ikezawa Z, Hashimoto K. The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. Br J Dermatol 2007;156:1083-4.
16. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, Halevy S, Davidovici BB, Mockenhaupt M, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? Br J Dermatol 2007;156:609-11.
17. Kano Y, Horie C, Inaoka M, Ishida T, Shiohara T. Herpes zoster in patients with drug-induced hypersensitivity syndrome/-DRESS. Acta Dermatol Venereol 2012;92:206-7.
18. Asano Y, Kagawa H, Kano Y, Shiohara T. Cytomegalovirus disease during severe drug eruptions: report of 2 cases and retrospective study of 18 patients with drug-induced hypersensitivity syndrome. Arch Dermatol 2009;145:1030-6.
19. Sakuma K, Kano Y, Fukuhara M, Shiohara T. Syndrome of inappropriate secretion of antidiuretic hormone associated with limbic encephalitis in a patient with drug-induced hypersensitivity syndrome. Clin Exp Dermatol 2008;33: 287-90.
20. Shiohara T, Kurata M, Mizukawa Y, Kano Y. Recognition of immune reconstitution syndrome necessary for better management of patients with severe drug eruption and those under immunosuppressive therapy. Allergol Int 2010;59: 333-43.
21. Leohloenya R, Meintjes G. Dermatologic manifestations of the immune reconstitution inflammatory syndrome. Dermatol Clin 2006;24:549-70.
22. Joly P, Janela B, Tetart F, Rogez S, Picard D, D'Incan M, et al. Poor benefit/risk balance of intravenous immunoglobulins in DRESS. Arch Dermatol 2012;148:543-4.
23. Takahashi R, Kano Y, Yamazaki Y, Kimishima M, Mizukawa Y, Shiohara T. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. J Immunol 2009; 182:8071-9.
24. Aota N, Shiohara T. Viral connection between drug rashes and autoimmune diseases: how autoimmune responses are generated after resolution of drug rashes. Autoimmune Rev 2009; 8:488-94.
25. Chen MR. Epstein-Barr virus, the immune system, and associated diseases. Front Microbiol 2011;2:5.
26. Niller HH, Wolf H, Ay E, Minarovits J. Epigenetic dysregulation of Epstein Barr virus latency and development of autoimmune disease. Adv Exp Med Biol 2011;711:82-102.
27. Tselis A. Epstein-Barr virus cause of multiple sclerosis. Curr Opin Rheumatol 2012;24:424-8.

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Suppression of polyI:C-inducible gene expression by EP3 in murine conjunctival epithelium

Keywords:
Prostaglandin E receptor 3 (EP3)
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Conjunctival epithelium

To the Editor,

We previously reported that EP3, a subtype of prostaglandin E_2 receptors (EP1-EP4), negatively regulates eosinophilic infiltration in murine experimental allergic conjunctivitis (EAC) induced by TLR3, which causes reduced eosinophilic conjunctival inflammation in TLR3/EP3 double knock-out (DKO) mice although in EP3-KO mice eosinophilic conjunctival inflammation is pronounced [1]. We also documented that in human conjunctival epithelial cells, the EP3 agonist suppressed the production of cytokines such as CXCL10, CXCL11, IL6, CCL5, TSLP, and MCP-1 induced by polyI:C, a TLR3 ligand [2]. EP3 was dominantly expressed in conjunctival epithelial cells [3], airway epithelial cells [4], and keratinocytes [5].

To examine the effects of EP3 against polyI:C-inducible gene expression in conjunctival epithelium we performed gene expression analysis of the polyI:C-stimulated conjunctival epithelium in wild-type, EP3-KO-, and EP3/TLR3 DKO mice.

Balb/c mice were purchased from CLEA (Tokyo, Japan). EP3/TLR3 DKO mice were produced by interbreeding EP3-KO- and TLR3-KO mice at Kyoto Prefectural University of Medicine [1]. All experimental procedures were approved by the Committee on Animal Research of Kyoto Prefectural University of Medicine, Kyoto, Japan.

For the *in vivo* analysis of murine conjunctival epithelial cells we prepared a 100 μg/ml polyI:C solution in 50% VISCOAT® (Alcon Laboratories Ltd, Fort Worth, TX)/PBS [6]. The polyI:C solution (each about10 μl) was injected subconjunctivally and dropped into the eyes as described elsewhere [6]. At 6 h after the injection, murine conjunctival tissues were resected and then murine conjunctival epithelium were detached and collected (Supplemental methods). Collected murine conjunctival epithelium almost consisted of epithelial cells (Supplemental Fig. 1). Quantitative RT-PCR was on an ABI-prism 7000 instrument (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The primers for the murine samples are shown in Supplemental Table 1. Microarray analysis was with Affymetrix GeneChip® mouse gene 1.0 ST arrays (Affymetrix, Santa Clara, CA, USA). Throughout the process we followed Affymetrix instructions (Supplemental Methods).

Using GeneChip® we first examined the comprehensive effects of gene expression in polyI:C-stimulated conjunctival epithelium of wild-type mice. We found that after 6-h stimulation, 31 transcripts were up-regulated more than 10-fold (Supplemental Table 2). Quantitative RT-PCR confirmed that 21 of the 31 transcripts (Cxcl10, Rsad2, Ifi205, Mx1, Cmpk2, Iigp1, Mx2, Iigp2, Ifit3, Gbp5, Cxcl11, H28, Slfn8, Plscr2, Slfn4, Usp18, Sectm1a, Oas2, Dhx58, Ccl5, Isg15) were significantly (>3-fold) up-regulated. Next, to identify the transcripts regulated by EP3 we compared the gene expression of these 21 transcripts in polyI:C stimulated conjunctival epithelium of wild-type and EP3-KO mice by quantitative RT-PCR. We found that all 21 transcripts were expressed significantly stronger in polyI:C stimulated conjunctival epithelium of EP3-KO mice (Fig. 1A). We also confirmed that the mRNA expression of these 21 transcripts was significantly reduced in polyI:C stimulated conjunctival epithelium of EP3/TLR3 DKO- compared to EP3-KO mice (Fig. 1A). *Ptger3* was almost undetectable in EP3-KO and EP3/TLR3-DKO mice as was TLR3 in EP3/TLR3-DKO mice (Fig. 1B).

GeneChip® analysis also showed that the number of 4 transcripts was more than 5 times greater in polyI:C stimulated conjunctival epithelium of EP3-KO- than wild-type mice although in wild-type mice these 4 transcripts were not significantly up-regulated after 6-h polyI:C stimulation (data not shown). Quantitative RT-PCR confirmed that the number of 2 of the 4 transcripts (Oas1g and Oas1a) was more than 100-fold higher in polyI:C stimulated EP3 KO- than wild-type mice (Fig. 1C).

We found that EP3 suppresses polyI:C-inducible genes in murine polyI:C stimulated conjunctival epithelium.

Of the 21 transcripts down-regulated by EP3, 13 (Cxcl10, Rsad2, Ifi205, Mx1, Iigp1, Mx2, Iigp2, Ifit3, Cxcl11, H28, Usp18, Oas2, and Isg15) are IFN-inducible genes. Our observations on EP3-KO mice suggest that Oas1g and Oas1a are markedly suppressed by EP3; they also are IFN-inducible genes and we posit that EP3 regulates the IFN-related response. It is of interest that there was no significant difference between wild-type and EP3/TLR3-DKO mice with respect to many of the 21 transcripts that were significantly up-regulated in EP3-KO mice. This suggests that polyI:C-inducible genes are regulated not only by TLR3 but also by other molecules

Abbreviations: EP3, prostaglandin E receptor 3; TLR3, toll-like receptor 3; EAC, experimental allergic conjunctivitis; DKO, double knock-out; TSLP, thymic stromal lymphopoietin; MCP-1, monocyte chemoattractant protein-1; polyI:C, polyinosinic:polycytidylic acid; Cxcl10, chemokine (C-X-C motif) ligand 10; Rsad2, radical S-adenosyl methionine domain containing 2; Ifi205, interferon activated gene 205; Mx1, myxovirus (influenza virus) resistance 1; Cmpk2, cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial; Iigp1, interferon inducible GTPase 1; Mx2, myxovirus (influenza virus) resistance 2; Iigp2, interferon inducible GTPase 2; Ifit3, interferon-induced protein with tetratricopeptide repeats 3; Gbp5, guanylate binding protein 5; Cxcl11, chemokine (C-X-C motif) ligand 11; H28, histocompatibility 28; Slfn8, schlafen 8; Plscr2, phospholipid scramblase 2; Slfn4, schlafen 4; Usp18, ubiquitin specific peptidase 18; Sectm1a, secreted and transmembrane 1A; Oas2, 2′-5′ oligoadenylatesynthetase 2; Dhx58, DEXH (Asp-Glu-X-His) box polypeptide 58; Ccl5, chemokine (C–C motif) ligand 5; Isg15, ISG15 ubiquitin-like modifier; Oas1g, 2′-5′ oligoadenylatesynthetase 1G; Oas1a, 2′-5′ oligoadenylatesynthetase 1A.

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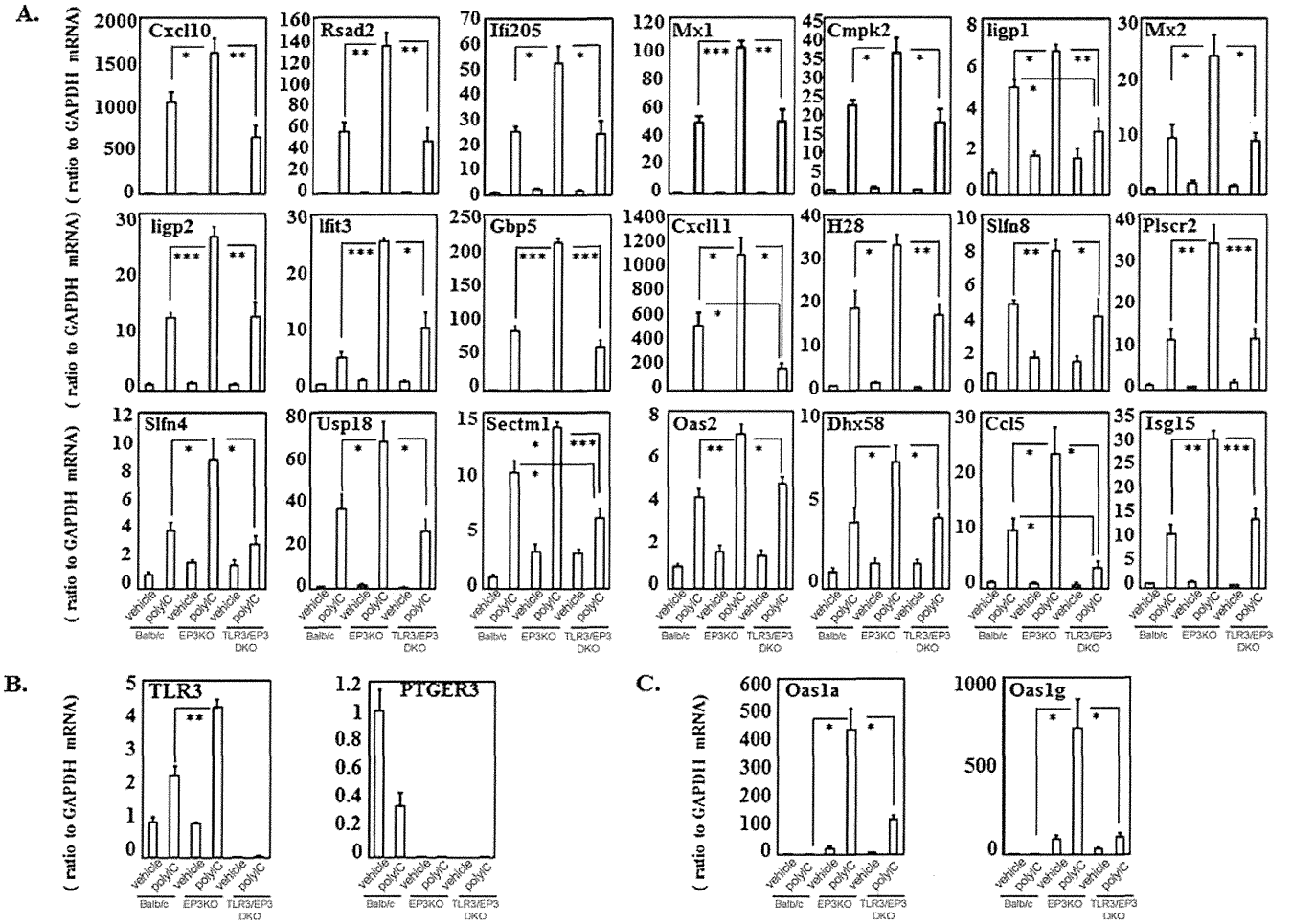

Fig. 1. Expression of transcripts induced by the polyI:C stimulation of conjunctival epithelium of wild-type-, EP3-KO-, and EP3/TLR3-KO mice. Quantification data were normalized to the expression of the housekeeping gene GAPDH. The *Y*-axis shows the increase in specific mRNA over unstimulated samples from wild-type mice. Data are representative of 3 separate experiments and show the mean ± SEM from one experiment carried out in 4 mice per group (*$p < 0.05$, **$p < 0.005$, ***$p < 0.005$).

such as MDA5 and RIG-I. We now know that EP3 suppresses polyI:C-inducible genes in polyI:C, a TLR3 ligand, stimulated conjunctival epithelium.

EP3 negatively regulates the eosinophilic infiltration of TLR3-induced murine EAC [1] and, EP3 and TLR3 were dominantly expressed in conjunctival epithelial cells [3,7]. In conjunctival epithelium EP3 suppresses polyI:C, a TLR3 ligand, inducible genes, suggesting that the conjunctival epithelium plays a critical role in the regulation of allergic conjunctivitis. Okuma et al. [8] recently reported that dysfunction of epithelial cells by the disruption of IκBζ induction elicits ocular surface inflammation via the activation of self-reactive lymphocytes, indicating that epithelial cells have an important role in the regulation of inflammation.

Elsewhere [1,8,9] we suggested that the pathogenesis of ocular surface inflammation such as Stevens–Johnson syndrome with severe ocular surface complications is associated with anomalies in innate immune reactions, especially reactions that involve epistatic interactions between TLR3 and EP3. We think that a lack of balance between TLR3 and EP3 is involved in triggering ocular surface inflammation [9].

In summary, we found that EP3 suppressed polyI:C, a TLR3 ligand, inducible genes in polyI:C stimulated murine conjunctival epithelium. Our findings suggest that EP3 and TLR3 in conjunctival epithelium play a critical role in regulating ocular surface inflammation.

Contributors

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Writing and review contributions to the manuscript: Mayumi Ueta.

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Financial relationship disclosure

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imlet.2013.08.010.

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References

[1] Ueta M, Tamiya G, Tokunaga K, Sotozono C, Ueki M, Sawai H, et al. Epistatic interaction between Toll-like receptor 3 (TLR3) and prostaglandin E receptor 3 (PTGER3) genes. Journal of Allergy and Clinical Immunology 2012;129:1413–6, e1411.
[2] Ueta M, Mizushima K, Yokoi N, Naito Y, Kinoshita S. Gene-expression analysis of polyI:C-stimulated primary human conjunctival epithelial cells. British Journal of Ophthalmology 2010;94:1528–32.
[3] Ueta M, Matsuoka T, Narumiya S, Kinoshita S. Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. Journal of Allergy and Clinical Immunology 2009;123:466–71.
[4] Kunikata T, Yamane H, Segi E, Matsuoka T, Sugimoto Y, Tanaka S, et al. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. Nature Immunology 2005;6:524–31.
[5] Honda T, Matsuoka T, Ueta M, Kabashima K, Miyachi Y, Narumiya S. Prostaglandin E(2)-EP(3) signaling suppresses skin inflammation in murine contact hypersensitivity. Journal of Allergy and Clinical Immunology 2009;124:809–18, e802.
[6] Ueta M, Kawai T, Yokoi N, Akira S, Kinoshita S. Contribution of IPS-1 to polyI:C-induced cytokine production in conjunctival epithelial cells. Biochemical and Biophysical Research Communications 2011;404:419–23.
[7] Ueta M, Hamuro J, Kiyono H, Kinoshita S. Triggering of TLR3 by polyI:C in human corneal epithelial cells to induce inflammatory cytokines. Biochemical and Biophysical Research Communications 2005;331:285–94.
[8] Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, et al. Enhanced apoptosis by disruption of the STAT3-IkappaB-zeta signaling pathway in epithelial cells induces Sjogren's syndrome-like autoimmune disease. Immunity 2013;38:450–60.
[9] Ueta M, Kinoshita S. Ocular surface inflammation is regulated by innate immunity. Progress in Retinal and Eye Research 2012;31:551–75.

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A Multicenter, Open-Label, 52-Week Study of 2% Rebamipide (OPC-12759) Ophthalmic Suspension in Patients With Dry Eye

SHIGERU KINOSHITA, SAKI AWAMURA, NORIHIRO NAKAMICHI, HIROYUKI SUZUKI, KAZUHIDE OSHIDEN, AND NORIHIKO YOKOI, ON BEHALF OF THE REBAMIPIDE OPHTHALMIC SUSPENSION LONG-TERM STUDY GROUP

• PURPOSE: To investigate the efficacy and safety of 2% rebamipide ophthalmic suspension administered 4 times daily for 52 weeks in patients with dry eye.

• DESIGN: Multicenter (17 sites), open-label, single-arm study.

• METHODS: A total of 154 patients with dry eye were enrolled in this study. After a 2-week screening period, patients received 2% rebamipide, instilled as 1 drop in each eye, 4 times daily for 52 weeks. The signs and symptoms measures were assessed at baseline, at weeks 2 and 4, and at every 4 weeks thereafter. The objective signs were fluorescein corneal staining score, lissamine green conjunctival staining score, and tear film break-up time, while subjective symptoms were dry eye–related ocular symptoms (foreign body sensation, dryness, photophobia, eye pain, and blurred vision). The safety variable was the occurrence of adverse events.

• RESULTS: For all objective signs and subjective symptoms, the scores significantly improved at week 2 compared with baseline ($P < .001$, paired t test). Interestingly, further improvements of those scores were observed at every visit up to week 52. No deaths were reported, yet serious adverse events that were not thought to be drug related were observed in 6 patients. The incidence of any of the adverse events did not markedly increase throughout the 52-week treatment period.

• CONCLUSION: The results of this study show that 2% rebamipide is effective in improving both the objective signs and subjective symptoms of dry eye patients for at least 52 weeks. In addition, 2% rebamipide treatment was generally well tolerated. (Am J Ophthalmol 2014;157: 576–583. © 2014 Published by Elsevier Ltd.)

DRY EYE IS DEFINED BY THE INTERNATIONAL DRY EYE Workshop as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface.[1] It is estimated that dry eye affects up to 35% of the human population worldwide,[2,3] and it has been reported that the disease is associated with deleterious effects on quality of life[3,4] and represents a potential substantial economic burden.[5] As the disease is often chronic and thus requires long-term treatment,[3] effective treatments must be tolerable and maintain their efficacy over a long-term period of use.

In healthy eyes, the ocular surface system functions to maintain and protect the refractive surface of the cornea.[6] In patients with dry eye, however, the disease reportedly disrupts components of the ocular surface, thus resulting in tear film instability.[1,6] The tear film can be destabilized by decreased tear production, delayed clearance, or altered tear composition, ultimately leading to inflammation and exacerbating tear film instability.[1]

Given the importance of the tear film in maintaining a healthy ocular surface, the role of mucins has been a recent focus in dry eye research. Mucins are either expressed on the membranes of ocular surface epithelia or secreted by conjunctival goblet cells, and play a role in lubrication and ocular defense.[7] Reduced amounts of mucins and changes in their distribution and glycosylation have been reported in patients with dry eye,[8,9] indicating that agents with the ability to increase mucin levels present a therapeutic option for treating the disease.

Rebamipide (Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan) is a mucosal protective agent with mucin secretagogue activity and is marketed in Japan as an oral drug for the treatment of gastric mucosal disorders and gastritis. It has been reported that rebamipide increases the production of mucin-like substances in the cornea and conjunctiva of a rabbit model in which ocular mucin was decreased by N-acetylcysteine,[10] and it has also been shown to increase the density of periodic acid–Schiff reagent–positive cells in the conjunctiva of normal rabbit eyes.[11] In addition, rebamipide reportedly increased MUC1 and MUC4 gene expression in human corneal epithelial cells, promoting glycoconjugate production, an indicator of mucin-like glycoprotein.[12] In a randomized, double-masked, placebo-controlled, 4-week phase II trial,[13] rebamipide ophthalmic suspension (1% and 2%) was found to be significantly more effective than the placebo in

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improving the fluorescein corneal staining score, the lissamine green conjunctival staining score, and tear film break-up time (TBUT), as well as being well tolerated by the patients and improving their subjective symptoms (foreign body sensation, dryness, photophobia, eye pain, and blurred vision), thus providing them with a positive overall treatment impression.[13] In addition, a phase III study found that 2% rebamipide was effective in improving the objective signs and subjective symptoms of dry eye.[14] Currently, rebamipide ophthalmic suspension has been marketed for the treatment of dry eye since 2012 in Japan.

The objective of this present study was to verify the efficacy and safety of 2% rebamipide ophthalmic suspension for the long-term treatment of dry eye, using objective and subjective outcome measures evaluated up to 52 weeks.

SUBJECTS AND METHODS

• STUDY DESIGN: This was a multicenter (17 sites), open-label, single-arm study of the efficacy and safety of 2% rebamipide treatment over a 52-week period. The study was performed in compliance with the tenets set forth in the Declaration of Helsinki. The study was conducted according to Good Clinical Practice Guidelines, and the study protocol and informed consent were reviewed and approved by the Institutional Review Boards at 17 Japanese clinical sites before initiation. In addition, informed consent was obtained from all potential patients prior to undergoing prescreening. The trial was registered at www.clinicaltrials.gov prior to patient enrollment (clinical trial identifier: NCT00818324).

The study design included a 2-week screening period for each patient to minimize the effects of any eye drops that had been used prior to the screening. During this screening period, preservative-free artificial tears (Soft Santear; Santen Pharmaceutical Co Ltd, Osaka, Japan) were administered 4 times daily. After a baseline examination, eligible patients underwent the administration of 1 drop of 2% rebamipide in each eye 4 times daily for 52 weeks; the use of the Soft Santear was not allowed during this period. The efficacy examinations were conducted at baseline, at weeks 2 and 4, and at every 4 weeks thereafter up to 52 weeks.

• PATIENTS: Subjects considered eligible for enrollment in the study were dry eye patients ≥20 years of age. Other inclusion criteria were as follows: (1) a score of ≥2 out of a maximum score of 4 for 1 or more dry eye–related ocular symptoms; (2) a fluorescein corneal staining score ≥3 out of a maximum score of 15; (3) a lissamine green conjunctival staining score ≥5 out of a maximum score of 18; (4) an unanesthetized Schirmer test value at 5 minutes of ≤5 mm or TBUT ≤5 seconds; and (5) a best-corrected visual acuity ≥20/100. Criteria 2-4 needed to be met in the same eye, but not necessarily in both eyes. Criteria 2 and 5 needed to be met at both the screening and baseline examinations, and criteria 1, 3, and 4 needed to be met at the screening examination.

Patients were excluded from the study if they had anterior ocular disease (such as blepharitis or blepharospasm), or if they were unable to discontinue the use of eye drops, currently had a punctal plug or had a punctal plug removed within 3 months prior to the screening examination, or had a history of surgical punctal occlusion. Other exclusion criteria included operation to the ocular surface within 12 months or intraocular surgery within 3 months prior to the screening examination.

The use of the following was prohibited during the study: oral rebamipide, ophthalmic drugs (with the exception of Soft Santear during the screening period), or contact lenses. In addition, ocular surgery or any other treatment affecting the dynamics of tear fluid, including its nasolacrimal drainage process, was prohibited during the study period. Eye drops, including all prescriptions and over-the-counter drugs, and other treatments for adverse events were permitted.

• OUTCOME MEASURES: *Efficacy*. Efficacy was evaluated with objective and subjective measures. The objective signs were fluorescein corneal staining score, lissamine green conjunctival staining score, and TBUT. The subjective symptoms were dry eye–related ocular symptoms (foreign body sensation, dryness, photophobia, eye pain, and blurred vision). All of these parameters were assessed at baseline and at weeks 2 and 4, then at every 4 weeks up to week 52, or at discontinuation.

For the fluorescein corneal staining, 5 μL of 2% fluorescein solution (provided by the sponsor) was instilled in the conjunctival sac as the patient was instructed to blink normally. Corneal staining was examined under standard illumination using slit-lamp microscopy. As per the NEI/Industry Workshop report,[15] the cornea was divided into 5 sections, each section was given a staining score from 0-3, and the total score was then calculated. The sponsor provided each investigator with a set of photographs of the fluorescein corneal staining to ensure standardization when scoring.

For the lissamine green conjunctival staining, 20 μL of 1% lissamine green solution (provided by the sponsor) was instilled in the conjunctival sac and the conjunctiva were divided into 6 sections.[15] Conjunctival staining was evaluated under low illumination by slit-lamp microscopy and scored from 0-3 for each section using photographs of lissamine green conjunctival staining provided by the sponsor. The total score was then calculated.

For the TBUT, 5 μL of 2% fluorescein solution was instilled in the conjunctival sac and slit-lamp microscopy was used for evaluation. The time from normal blinking to first appearance of a dry spot in the tear film was measured 3 times.

Dry eye–related ocular symptoms, such as foreign body sensation, dryness, photophobia, eye pain, and blurred vision, were examined by questioning each patient. Answers to the questions regarding the severity of those symptoms were scored according to the following criteria: 0, no symptoms;

TABLE 1. Disposition of Dry Eye Patients Treated With 2% Rebamipide (OPC-12759) Ophthalmic Suspension in This Multicenter, Open-Label, 52-Week Study

	2% Rebamipide n (%)
Number of patients treated	154
Number of patients completing treatment	127 (82.5)
Number of patients discontinuing treatment	27 (17.5)
-Discontinuation owing to occurrence of adverse events	22 (14.3)
-Discontinuation owing to the patient's wish	1 (0.6)
-Discontinuation owing to protocol deviation	2 (1.3)
-Discontinuation for other reason	2 (1.3)

TABLE 2. Demographics and Other Baseline Characteristics of Dry Eye Patients Treated With 2% Rebamipide (OPC-12759) Ophthalmic Suspension in This Multicenter, Open-Label, 52-Week Study

	2% Rebamipide (N = 154) n (%)
Sex	
Male	15 (9.7)
Female	139 (90.3)
Age	
20-49 years	37 (24.0)
50-64 years	53 (34.4)
≥65 years	64 (41.6)
Main cause or primary disease of dry eye	
Primary Sjögren syndrome	21 (13.6)
Secondary Sjögren syndrome	5 (3.2)
Stevens-Johnson syndrome	5 (3.2)
Unknown	123 (79.9)
Fluorescein corneal staining score[a]	
3-6	91 (59.1)
7-9	36 (23.4)
10-15	27 (17.5)
Dry eye–related ocular symptoms[a]	
Foreign body sensation	120 (77.9)
Dryness	145 (94.2)
Photophobia	98 (63.6)
Eye pain	90 (58.4)
Blurred vision	88 (57.1)

[a]At baseline.

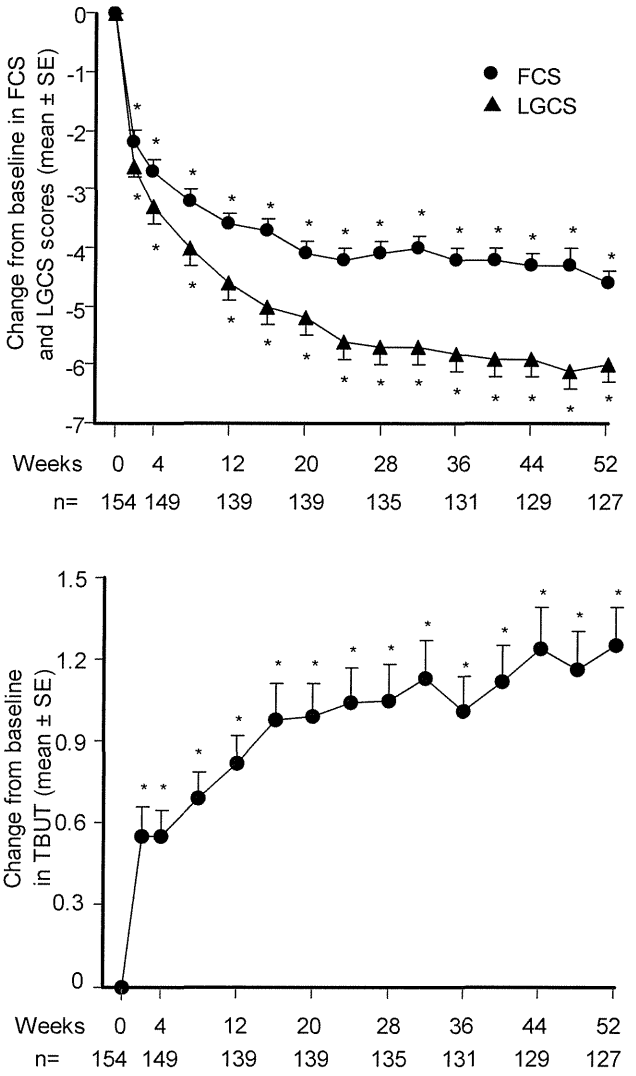

FIGURE 1. Change from baseline in fluorescein corneal staining scores and lissamine green conjunctival staining scores (Top) and tear film break-up time (Bottom) with 2% rebamipide (OPC-12759) ophthalmic suspension treatment in dry eye patients over 52 weeks. The scores of fluorescein corneal staining, lissamine green conjunctival staining, and tear film break-up time significantly improved at week 2 compared with baseline, and further improvements were observed at almost every visit up to 52 weeks. *$P < .001$ compared with respective baseline, paired t test. FCS = fluorescein corneal staining; LGCS = lissamine green conjunctival staining; SE = standard error; TBUT = tear film break-up time.

1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms.

Safety. The safety variable was the occurrence of adverse events, determined at each visit by assessment of physical signs and symptoms, external eye examination and slit-lamp microscopy, visual acuity, intraocular pressure, funduscopy, and clinical laboratory tests. Clinical laboratory tests included hematology, biochemistry, and urinalysis.

• STATISTICAL ANALYSIS: The efficacy and safety analysis sets comprised patients who received 2% rebamipide at least once. Missing efficacy and safety data were treated as missing data. The eye in which objective efficacy endpoints were analyzed was determined as follows: (1) if only 1 eye met the inclusion criteria, this eye was used; (2) if both eyes met the inclusion criteria, the eye with the higher fluorescein corneal staining score was used; (3) if both eyes had