Analysis of Current Treatments and Deceased Cases of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Japan

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To examine the current treatments for Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Japan, we retrospectively analyzed reports of 43 cases of SJS and 54 cases of TEN published in medical journals from 2000 to 2005. Corticosteroid was administered systemically in most cases, and methylprednisolone (mPSL) pulse therapy (1,000mg/day) or mini pulse (less than 600mg/day) was selected for 14 cases (32.6%) of SJS and 28 cases (51.9%) of TEN. Combinations of plasmapheresis or high-dose immunoglobulin therapy with corticosteroid therapy were performed in 5 cases of TEN and 3 cases of TEN. In 2 cases of TEN in which the symptoms continued to progres in spite of using corticosteroids and high-dose immunoglobulin therapy, plasmapheresis was performed additionally and was effective in their recovery. This suggests that the combination of these 3 treatments may be useful in seriously ill patients. The mortality rate of patients with SJS was 2.3%, and with TEN was 7.4%.

We also reviewed the deceased cases published in medical journals from 2000 to 2005. One case of SJS and 4 cases of TEN were reported.

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Key words : prognosis, Stevens-Johnson syndrome, toxic epidermal necrolysis, treatment

TABLE. Intravitreal Prednisolone Sodium Succinate Injections for Persistent Diabetic Macular Edema: Qualitative (FAG) or Quantitative (OCT) Assessment of CME at 3 Months, Whereupon the Decision to Re-treat was Based

	CME at 3 Months: FAG or OCT	CME at 6 Months: FAG or OCT
Worse	2 (10.5%)	3 (15.8%)
Stabile	3 (15.8%)	3 (15.8%)
Decreased	14 (73.7%)	13 (68.4%)

CME = cystoid macular edema; FAG = fluorescein angiography; OCT = ocular coherence tomography.

Need for retreatment was based on fluorescein angiographic or OCT evidence of persisting (eg "worse or stabile") macular edema at 3-month follow-up. The pre- and posttreatment angiograms at 6 months were reviewed in a masked fashion.

months mean visual acuity improvement was 5.4 ETDRS letters. Visual acuity at six months was stabilized or improved in 89% of the eyes. Two (11%) of the 19 eyes had a regression in visual acuity at six months compared with preoperatively, although both eyes showed a visual improvement at three months. For all eyes, mean intraocular pressure before injection was 15.6 (± 3.1) mm Hg, and at six months postoperative 14.3 (± 2.9) mm Hg. We did not observe intraocular pressures that exceeded 22 mm Hg in any of the eyes during follow-up, and no antiglaucoma medication was needed. Retreatment rate was 1.3 injections per eye after a mean period of 13.2 weeks. Macular edema decreased in 13 eyes (69.4%) (Figure 2, Table). No other adverse events, such as endophthalmitis, vitreous hemorrhage, or retinal detachment occurred.

In summary, mean visual acuity improvement after intravitreal prednisolone sodium succinate was statistically significant compared with preoperative visual acuity up to six months postoperatively. Prednisolone sodium succinate has glucocorticoid activity, but we encountered no significant increase in intraocular pressure and no other adverse events in the small group of studied eyes during follow-up, although no risk factors for glaucoma (i.e., family history, myopia greater than 5 diopters, or a history of collagen vascular disease) were present in any of the study patients. Perhaps this may be attributable to the fact that, in contrast with the crystalline cortisone of triamcinolone acetonide, prednisolone sodium succinate is injected as a transparent solution. Although the number of eyes in this pilot study was limited, results suggest that intravitreal injection of the transparent solution of prednisolone sodium succinate may be a safe and good alternative for triamcinolone acetonide in eyes with macular edema. Because the follow-up in this study was short, long-term efficacy of intravitreal prednisolone sodium succinate needs further analysis.[5]

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A Comparison Between Cultivated and Conventional Limbal Stem Cell Transplantation for Stevens-Johnson Syndrome

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PURPOSE: To compare the resolution of inflammation and long-term results of cultivated and conventional limbal stem cell transplantation (LSCT) in a patient with Stevens-Johnson syndrome (SJS).

DESIGN: Interventional case report.

METHODS: A 32-year-old man with SJS and bilateral total limbal stem cell deficiency underwent cultivated LSCT in the right eye, followed by conventional LSCT in the left eye three weeks later. The postoperative medication included dexamethasone 0.1% and ofloxacin 0.3% eyedrops and a tapering dose of systemic corticosteroid, cyclosporine, and cyclophosphamide. Tear samples were collected and analyzed for interleukin (IL) 8 levels.

RESULTS: Complete corneal epithelialization was achieved 48 hours after cultivated LSCT, compared with three

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weeks after conventional LSCT. Ocular inflammation and IL-8 levels decreased more rapidly in the eye with cultivated LSCT. Four years after surgery, more severe corneal scarring and opacification were noted in the conventional LSCT eye.

CONCLUSIONS: Cultivated LSCT resulted in a better clinical result and vision, with less stromal scarring compared with conventional LSCT. (Am J Ophthalmol 2007; 143:178–180. © 2007 by Elsevier Inc. All rights reserved.)

SEVERE OCULAR SURFACE DISEASE AND LIMBAL STEM cell destruction arising from Stevens-Johnson syndrome (SJS) remain a major clinical challenge for ophthalmologists because these conditions do poorly with conventional corneal transplantation. Limbal stem cell transplantation (LSCT) helps to regenerate the corneal epithelium in these severely damaged eyes.[1] More recently, cultivated LSCT has also demonstrated promising results.[2,3] However, most of these studies have been noncomparative case series. To date, to our knowledge, there has been no report comparing the relative efficacy of conventional and cultivated LSCT. We describe a comparison of the long-term efficacy of cultivated and conventional LSCT in a patient with SJS and compare the resolution of ocular inflammation by cytokine analysis.[4]

A 32-year-old man with SJS developed bilateral total limbal stem cell deficiency with subtotal persistent epithelial defects, corneal conjunctivalization, and neovascularization. His visual acuity for both eyes was 20/40, and both eyes had severe persistent inflammation. Cultivated LSCT was performed in the right eye three months after disease onset. Limbal epithelial cells of donor tissue from Northwest Lion Eye Bank were enzymatically disaggregated and cultured on a denuded amniotic membrane, as previously described.[3] Surgery involved removal of the corneal pannus and scarred perilimbal tissue, application of mitomycin-C 0.04%, and transplantation of the cultivated corneal epithelial sheet.[3] Postoperative medication included dexamethasone 0.1% and ofloxacin 0.3% eyedrops and a tapering dose of systemic corticosteroid, cyclosporine, and cyclophosphamide. Three weeks later, the patient underwent conventional LSCT in the left eye. Excision of the diseased tissue and mitomycin C application was similarly performed, followed by transplantation of four quadrants of limbal allografts onto the recipient limbal region. A similar postoperative medication regime was used. Tears collected before and after surgery were analyzed for interleukin (IL)-8 levels with an enzyme-linked immunosorbent assay test (ELISA) kit.[4]

Complete epithelialization was achieved 48 hours after cultivated LSCT, compared with three weeks after conventional LSCT. Ocular inflammation and IL-8 levels were noted to decrease more rapidly in the eye with cultivated LSCT compared with the conventional LSCT eye (Figures 1 and 2). The eye with conventional LSCT developed greater stromal scarring and vascularization, whereas the cultivated LSCT eye remained reasonably clear (Figure 1, Bottom). Four years after surgery, visual acuity was 20/30 in the right eye and 20/100 in the left eye, with more severe corneal scarring and opacification noted in the left eye. The corneal epithelium remained fairly stable in both eyes.

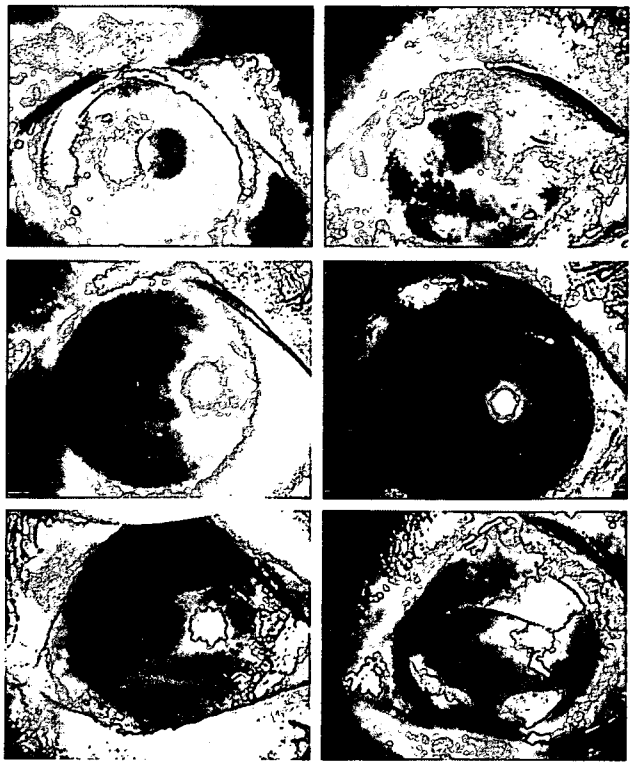

FIGURE 1. Preoperative and postoperative appearance of cultivated (left) and conventional limbal stem cell transplantation (right). (Top) Preoperative appearance. (Middle) Postoperative appearance at two months. (Bottom) Postoperative appearance at four years.

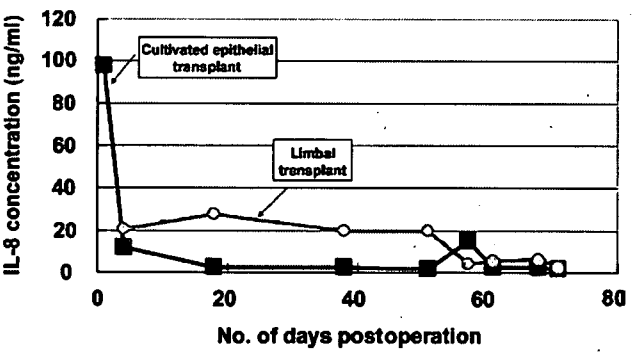

FIGURE 2. Cultivated and conventional limbal stem cell transplantation, pre- and postoperative interleukin (IL)-8 concentration in tears. A faster decrease in IL-8 levels was noted after cultivated epithelial transplantation compared with conventional limbal transplantation.

We compared the clinical results of conventional and cultivated LSCT in the same patient with SJS, thereby eliminating any interpatient variability. Cultivated LSCT resulted in a better clinical result and vision, with less stromal scarring compared with conventional LSCT. IL-8, a proinflammatory cytokine, was found to decrease more rapidly in the eye with cultivated LSCT. The almost immediate epithelialization achieved by the cultivated epithelial sheet, together with the use of an amniotic membrane substrate, may have contributed to faster ocular rehabilitation, with reduced inflammation and corneal scarring. Reduced stromal scarring allows repeat transplantation to be easily performed, without requiring further lamellar dissection in an already compromised cornea.[5] This study provides valuable information regarding the effective use of cultivated LSCT for the treatment of total limbal stem cell deficiency in SJS.

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Aspergillus fumigatus Colonization of Punctal Plugs

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PURPOSE: Punctal plugs are used in patients with dry eye syndrome to preserve the tears. In this report, I present two cases of *Aspergillus fumigatus* colonization of punctal plugs.

DESIGN: Observational series of two cases.

METHODS: Approval was obtained from the institutional review board. Two men aged 29 and 31 years developed black spots inside the hole of punctal plug, which looked like eyeliner deposits. The deposits inside the hole of the plug in each patient were removed and cultured.

RESULTS: Cultures of the two punctal plugs black deposits grew *A fumigatus*. Bacterial cultures were negative.

CONCLUSIONS: Colonization of the punctal plug hole with *A fumigatus* was observed in two cases. It is recommended that punctal plugs be removed in patients undergoing refractive or intraocular procedures or in patients who are receiving topical corticosteroids. Current punctal plugs should be redesigned to avoid the presence of an inserter hole. (Am J Ophthalmol 2007;143:180–181. © 2007 by Elsevier Inc. All rights reserved.)

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PUNCTAL PLUGS ARE USED FOR THE MANAGEMENT OF dry eye syndrome.[1–4] The plugs help in preservation of tears and are indicated in certain cases of laser in situ keratomileusis and contact lens intolerance. Dry eye syndrome may compromise the ocular surface, leading to corneal erosions that may predispose the patient to microbial keratitis. Punctal plugs may cause localized entrapment and colonization of bacteria and fungi. Most of the punctal plugs have a central hole where the inserter pin is fitted for insertion of the plug. The inserter pin is withdrawn, leaving an open cavity. Colonization of organisms may occur inside the plug hole.[5] The main purpose of this report is to present two cases of *Aspergillus fumigatus* growth inside the punctal plug hole.

• CASE 1: A 29-year-old man who presented with history of foreign body sensation and mucoid discharge of two years' duration. He was found to have normal vision and reduced tearing. He had bilateral pinguecula and no corneal staining. The rest of the examination was normal. The patient was diagnosed as having dry eye syndrome, and the lower puncta were occluded by Eagle FlexPlug (EagleVision, Memphis, Tennessee, USA). After insertion of the punctal plug, the patient's symptoms improved. Three months after the insertion of the plug, he came for a follow-up examination and was found to have black deposits in the punctum of the right lower lid (Figure 1). The deposits in the hole of the punctal plug were removed and cultured onto Sabouraud agar, blood agar, chocolate agar, and thioglycolate. The culture netted a pure growth of *A fumigatus*. There was no bacterial growth. The patient was followed, and he had no canaliculitis and no evidence of conjunctivitis or keratitis.

this time frame because this procedure is associated with complications such as lens damage, patient discomfort, and theoretical risk of increased infection. Additionally, it may not be necessary for the patient to remain at the clinic immediately after intravitreal injection for an IOP check.

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Strong Association Between HLA-A*0206 and Stevens-Johnson Syndrome in the Japanese

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PURPOSE: To investigate the association between HLA class I antigens and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) with ocular complications in Japanese.

DESIGN: Case-control study.

METHODS: We examined the histocompatibility antigen genes HLA-A, -B, and -C of 40 Japanese SJS/TEN patients with ocular complications and 113 healthy Japanese volunteers by polymerase chain reaction amplification and subsequent hybridization with sequence-specific oligonucleotide probes (PCR-SSO).

RESULTS: We clarified that HLA-A*0206 is strongly associated with SJS/TEN with ocular complications in the Japanese.

CONCLUSIONS: Because this finding is completely different from data reported elsewhere on Taiwanese Han Chinese patients and Caucasian patients, it suggests strong ethnic differences in the HLA-SJS association and points to the need for studies in other ethnic populations in order to obtain a global picture. (Am J Ophthalmol 2007;143: 367–368. © 2007 by Elsevier Inc. All rights reserved.)

STEVENS-JOHNSON SYNDROME (SJS) AND TOXIC EPIDERmal necrolysis (TEN) are acute-onset mucocutaneous diseases induced by infectious agents and/or inciting drugs. Based on a large international case-control study, SJS and TEN are considered as severity variants of a single entity[1]; developing acute exanthema that progresses to limited (SJS) or more widespread (TEN) blistering and erosion of the skin and mucous membranes. Although rare, these reactions carry high morbidity and mortality rates. Ophthalmologists recognize the serious ocular complications leading to severe, lifelong visual dysfunction. Conjunctival invasion into the cornea attributable to corneal epithelial stem cell deficiency progresses despite healing of the skin lesions, and corneal opacity, neovascularization, symblepharon, ankyloblepharon, and in some instances, keratinization, appears on the ocular surface at the chronic stage. Interestingly, we observed that more than 95% of three patients out of 61 SJS/TEN with ocular complications had lost their fingernails in the acute stage and transformed nails often continue even after healing of the skin lesions. The reported incidence of ocular complications is 50% to 69%. The pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established, although the involvement of immune mechanisms and an altered drug metabolism have been suggested. Whatever the pathogenetic events, the extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility.

We studied the histocompatibility antigen genes HLA-A, -B, and -C of Japanese SJS/TEN patients with ocular complications. The study was approved by the institutional review board, and consent was obtained from all participants in written form. The diagnosis of SJS/TEN was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites including the

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TABLE. Frequency of HLA Class I Alleles in Patients with Stevens-Johnson Syndrome (SJS)/Toxic Epidermal Necrolysis (TEN)

HLA Allele	SJS/ TEN with Ocular Complications		Control Subjects		*P* value (χ^2)	Corrected *P*#	Odds Ratio
	No.	%	No.	%			
Carrier frequency	(n = 40)		(n = 113)				
A*0206	19/40	47.5%	17/113	15.0%	0.00003	<0.0005	5.1
A*1101	1/40	2.5%	23/113	20.4%	0.0076	NS	-
Gene frequency	(n = 80)		(n = 226)				
A*0206	21/80	26.3%	19/226	8.4%	0.00005	<0.0005	3.9
A*1101	1/80	1.3%	26/226	11.5%	0.0055	<0.05	0.1

#: Corrected *P* is *P* after correction for multiple (9) comparisons.

ocular surface. Forty patients and 113 healthy Japanese volunteers were genotyped by polymerase chain reaction amplification and subsequent hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial typing kits (WAK Flow, Wakunaga, Hiroshima, Japan). All participants and volunteers were Japanese residing in Japan.

We show that in the Japanese, among HLA-class I (HLA-A, -B, and -C), HLA-A*0206 was strongly associated with SJS/TEN with ocular complications ($Pc < .0005$, OR = 5.1) and HLA-A*1101 was inversely associated (Table). On the other hand, HLA-B, HLA-C, and other HLA-A alleles were not significantly associated with SJS/TEN.

A report from the United States showed that the HLA-B12 (HLA-Bw44) antigen was considerably increased in Caucasian SJS patients with ocular involvement.[2] Analyses of SJS/TEN patients in France also disclosed an association with HLA-B12 (HLA-Bw44).[3] In our study population, we did not find such an association with HLA-B12, probably because in Caucasians, the HLA-B12 antigen is primarily coded by HLA-B*4402, whereas in Japanese, it is almost exclusively coded by a different allele, such as HLA-B*4403.[4] A Taiwanese study[5] reported a very strong association between carbamazepine-induced SJS in Han Chinese patients and the HLA-B*1502 allele. However, Lonjou and associates[6] countered that this allele is not a universal marker for SJS and that ethnicity plays a role. While HLA-B*1502 was considerably increased in the Han Chinese patients with carbamazepine-induced SJS,[6] this allele is almost completely absent in the Japanese population. Conversely, HLA-A*0206 associated with Japanese SJS/TEN is absent in Caucasians and less frequent in Southern Han Chinese.[6] Therefore, HLA-A*0206 may be related to ethnicity in Japanese. Our findings suggest strong ethnic differences in the HLA-SJS/TEN association and point to the need for studies in other ethnic populations to obtain a global picture.

Because SJS/TEN is a rare condition that is probably associated with a complex genetic inheritance background, it is possible that specific combinations of genes are required for the onset of the disease. The strong association of specific HLA antigens with SJS with ocular complications may be a clue to understanding its basic pathobiology and enables us to develop a reliable test for predicting subjects susceptible to SJS with ocular complications.

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New Grading System for the Evaluation of Chronic Ocular Manifestations in Patients with Stevens–Johnson Syndrome

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Purpose: To evaluate and grade the extent and severity of chronic ocular manifestations in Stevens–Johnson syndrome (SJS).

Design: Prospective multicenter case series.

Participants: We enrolled 73 patients (138 eyes) with SJS seen between April 2003 and March 2005 at 3 tertiary referral centers.

Methods: Patients with a confirmed history of SJS and chronic ocular complications that persisted for at least 1 year from the onset of SJS were included. Their detailed medical history and ophthalmic examination results were recorded on an itemized data collection form. Complications were categorized as corneal, conjunctival, and eyelid complications, and 13 components were evaluated and graded on a scale from 0 to 3 according to their severity.

Main Outcome Measures: These were broadly classified as corneal (superficial punctate keratopathy, epithelial defect, loss of the palisades of Vogt, conjunctivalization, neovascularization, opacification, keratinization), conjunctival (hyperemia, symblepharon formation), and eyelid (trichiasis, mucocutaneous junction involvement, meibomian gland involvement, punctal damage) complications.

Results: The most severely affected complication components were loss of the palisades of Vogt (114 eyes; 82.6%) and meibomian gland involvement (102 eyes; 73.9%). Visual acuity in 74 of the 138 eyes (53.6%) was worse than 20/200. The severity of corneal, conjunctival, and eyelid complications was significantly correlated with visual loss. All 13 complications were correlated significantly with logarithm of the minimum angle of resolution (logMAR) visual acuity; the correlation coefficient (R) ranged from 0.359 to 0.810 ($P<0.0001$); for corneal epithelial defects, R was 0.169 ($P = 0.0473$). Eyes with a higher total score for the 3 complication categories had poorer vision ($R = 0.806$; $P<0.0001$). Multivariate regression analysis showed that corneal neovascularization, opacification, keratinization, and cataracts significantly affected logMAR visual acuity ($P<0.0001$, $P<0.0001$, $P = 0.0142$, $P = 0.0375$, respectively).

Conclusions: The authors describe a new method for grading the extent and severity of ocular involvement in patients with SJS and demonstrate that the severity of ocular involvement is correlated significantly with the final visual outcome. This new grading system provides a more objective method for evaluating SJS patients and may be adapted for use in other cicatricial ocular surface diseases. *Ophthalmology 2007;114:1294–1302 © 2007 by the American Academy of Ophthalmology.*

Stevens–Johnson syndrome (SJS) is an acute, self-limiting disease of the skin and mucous membranes that predisposes patients to life-threatening complications such as sepsis, respiratory dysfunction, and multiorgan failure. In the acute stage, more than 50% of patients experience ocular complications ranging from minimal (e.g., mild conjunctival hyperemia) to very severe (e.g., corneal melting and perforation).[1–4] Inflammation and epithelial erosion of the ocular surface often persist beyond the acute stage and the resolu-

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tion of skin eruptions, leading to ocular complications and scarring in the chronic stage. Severe ocular surface disease arising from SJS encompasses a spectrum of ocular manifestations and complications that often is associated with significant visual morbidity. Visual impairment and ocular discomfort continue throughout life and patients usually require long-term medication for disease control.

Over the past 40 years, it has been widely accepted that erythema multiforme (EM), SJS, and toxic epidermal necrolysis (TEN) are part of a single EM spectrum.[5–7] However, because no clear diagnostic criteria have been established, reaching a definitive diagnosis can be difficult. Roujeau,[6] who performed a retrospective analysis of the type and distribution of skin lesions and the extent of epidermal detachment, concluded that EM major (EMM) and SJS were 2 separate clinical entities that differed with respect to histopathologic changes and cause. A large international case-control study, called the Severe Cutaneous Adverse Reaction study, prospectively evaluated the validity of this clinical distinction; its results strongly support the hypothesis that EMM is different from SJS and TEN, and that SJS and TEN are severity variants of a single entity.[5] The classification was based on the clinical appearance and pathologic results of skin lesions present in the acute stage. However, patients often seek treatment from ophthalmologists in the late stage of the disease with chronic cicatricial complications, after resolution of the dermatologic changes, and it can be difficult to elicit the original clinical manifestations used to distinguish between EMM and SJS or TEN from patients seen many years after disease onset. Therefore, from the ophthalmologist's perspective, ocular surface diseases arising from EM, SJS, or TEN often are regarded collectively as SJS.

Corneal transplantation in SJS patients with severe ocular surface disease is associated with a poor prognosis. Persistent epithelial defects occurring after penetrating or lamellar keratoplasty often progress to corneal melting and perforation. Transplanted limbal stem cells or keratoepithelioplasty in these chronically inflamed eyes often elicit graft rejection and loss of donor epithelial cells, resulting in progressive conjunctivalization, scarring, and visual loss.[8,9] Over the past decade, new ocular surface reconstructive procedures such as amniotic membrane and cultivated epithelial transplantation have yielded promising results for the treatment of SJS.[10,11] However, despite its potentially devastating nature and the increasing indications for ocular reconstructive surgery, there is currently no standardized method for evaluating the spectrum of ocular manifestations and the severity of ocular complications in this blinding disease.

The aims of this study were to elucidate the profile of chronic ocular manifestations in SJS patients and to develop an objective method for grading the extent and severity of ocular complications in patients with cicatricial ocular surface diseases. Three large tertiary referral ophthalmic centers participated in this multicenter study; to our knowledge, it represents the largest series of SJS patients with ophthalmic complications studied to date. Because it provides a common platform for the discussion and management of these patients, this study has important clinical implications for the diagnosis, treatment, and the prediction of visual outcomes in patients with SJS.

Patients and Methods

Patients

The 3 ophthalmic centers that participated in this multicenter study are Kyoto Prefectural University of Medicine, Keio University, and National Tokyo Medical Center. All patients with chronic ocular complications from SJS who were referred to these centers between April 2003 and March 2005 were evaluated prospectively in this study. Patients with a confirmed history of SJS and chronic ocular complications that persisted for at least 1 year from the onset of SJS were included. The diagnosis of SJS was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites including the ocular surface. Eyes with a past history of ocular surface surgery were excluded from this study. The study was approved by the ethics committee and institutional review boards of each institution; the guidelines of the Declaration of Helsinki in Biomedical Research Involving Human Subjects were followed, and written informed consent was obtained from each patient.

The symptomatology, physical findings, detailed ophthalmic examination results, and ocular complications were recorded on an itemized data collection form. The detailed ophthalmic examination included an assessment of visual acuity, tonometry, slit-lamp examination, fluorescein staining, and anterior segment photography. A careful drug history also was obtained by the attending physician. A drug was considered a possible etiologic agent if it had been taken shortly before the onset of symptoms, that is, within 2 weeks of disease onset. If the reaction showed signs of regression during the continued administration of the drug, a causal relationship was considered unlikely.

Classification and Grading of Ocular Involvement

We considered 13 components of 3 categories of ocular complications to be important in the assessment of the 138 eyes; each component was graded on a scale from 0 to 3, depending on the severity of involvement. The complications were classified broadly as corneal complications, comprised of superficial punctate keratopathy (SPK), epithelial defect, loss of the palisades of Vogt (POV), conjunctivalization, neovascularization, opacification, and keratinization components; conjunctival complications with hyperemia and symblepharon formation as the components; and eyelid complications consisting of trichiasis, mucocutaneous junction involvement, meibomian gland involvement, and punctal damage as the evaluated components. The following classification and grading systems were used to evaluate the nature of the ocular complications in these patients.

Corneal Complications

Severity of Superficial Punctate Keratopathy. We used fluorescein staining and a simplified method of Miyata et al[12] to grade SPK based on the area and density of the lesions. The area was graded as A0 when there was no punctate staining and as A1, A2, or A3 when the area occupied less than one third, one third to two thirds, or more than two thirds of the cornea, respectively. Density was graded as D0 when there was no punctate staining and as D1,

D2, or D3 when density was sparse, moderate, or high and the lesions overlapped, respectively. Although Miyata et al used the sum of the grades assigned to the area and density to obtain the final grade for the eye, we simplified their grading system and assigned scores of 0 through 3: A1D1 was scored as 0; A1D2 or A2D1 was scored as 1; A1D3, A2D2, or A3D1 was scored as 2; and A2D3, A3D2, or A3D3 was scored as 3.

Corneal Epithelial Defect. The extent of corneal epithelial defect was scored from 0 through 3, where 0 = no epithelial defect, 1 = epithelial defect involving less than one quarter of the corneal surface, 2 = defect involving one quarter to one half, and 3 = defect involving more than one half of the corneal surface.

Loss of the Palisades of Vogt. The extent of the loss of the limbal POV was graded from 0 through 3, where 0 = presence of the entire POV, 1 = loss of less than half of the entire circumference of POV, 2 = loss of more than half of the entire circumference of POV, and 3 = total loss of POV.

Conjunctivalization. The extent of conjunctivalization was graded clinically from 0 through 3 as follows: 0 = absence of conjunctivalization, 1 = conjunctivalization involving less than one quarter of the corneal surface, 2 = conjunctivalization involving one quarter to one half, and 3 = conjunctivalization involving more than one half of the corneal surface (Fig 1).

Corneal Neovascularization. The extent of corneal neovascularization was scored from 0 through 3, where 0 = no neovascularization, 1 = neovascularization confined to the corneal periphery, 2 = neovascularization extending up to the pupil margin, and 3 = neovascularization extending beyond the pupil margin into the central cornea (Fig 1). In eyes where significant opacification or extensive symblepharon formation made it difficult to evaluate corneal neovascularization, a score of 3 was assigned.

Corneal Opacification. The severity of corneal opacification was graded from 0 through 3, where 0 = clear cornea with iris details clearly visualized, 1 = partial obscuration of the iris details, 2 = iris details poorly seen with pupil margin just visible, and 3 = complete obscuration of iris and pupil details (Fig 1).

Corneal Keratinization. The extent of keratinization was graded from 0 through 3, where 0 = no corneal keratinization, 1 = keratinization involving less than one quarter of the corneal surface, 2 = keratinization involving one quarter to one half, and 3 = keratinization involving more than one half of the corneal surface (Fig 1).

Conjunctival Complications

Conjunctival Hyperemia. Conjunctival hyperemia was graded from 0 through 3 based on the following clinical features: 0 = absence of hyperemia, 1 = mild (mild or sectoral engorgement of the conjunctival vessels), 2 = moderate (diffuse engorgement of the conjunctival vessels), and 3 = severe hyperemia (significant engorgement of the conjunctival vessels).

Symblepharon Formation. The extent of symblepharon formation was scored from 0 through 3, where 0 = no symblepharon, 1 = symblepharon formation involving only the conjunctival surface, 2 = symblepharon formation involving less than half of the corneal surface, and 3 = symblepharon formation involving more than half of the corneal surface (Fig 1).

Eyelid Complications

Trichiasis. The extent of trichiasis for the total area of the upper and lower eyelids combined was scored as 0 through 3, where 0 = no trichiasis, 1 = trichiasis involving less than one quarter of the lid margin, 2 = trichiasis involving one quarter to one half of the lid margin, and 3 = trichiasis involving more than one half of the lid margin.

Mucocutaneous Junction Involvement. The severity of mucocutaneous junction involvement was scored from 0 through 3, where 0 = normal mucocutaneous junction, 1 = mild irregularity of the mucocutaneous junction, 2 = moderate irregularity of the mucocutaneous junction, and 3 = severe irregularity of the mucocutaneous junction (Fig 2). Fluorescein staining of the conjunctiva was helpful in evaluating the involvement of the mucocutaneous junction. Normal mucocutaneous junction showed the linear staining at the end of the conjunctiva, and either mild, moderate, or severe irregularity of this line was observed in the eyes with mucocutaneous junction involvement. In eyes where significant keratinization of the lid margin or extensive symblepharon formation made it difficult to evaluate mucocutaneous junction involvement, a score of 3 was assigned.

Meibomian Gland Involvement. The severity of meibomian gland involvement was determined clinically by the nature of the meibomian gland secretion expressed manually at the center of the upper lid and was scored from 0 through 3, where 0 = clear oily fluid expressed, 1 = yellowish-white oily fluid expressed, 2 = thick cheesy material expressed, and 3 = inability to express any fluid from the meibomian glands.

Punctal Involvement. Punctal damage and occlusion were graded from 0 through 3, where 0 = normal patent puncta, 1 = iatrogenic punctal occlusion (e.g., punctal plugs or suture), 2 = either superior or inferior puncta occluded by scarring, and 3 = both superior and inferior puncta occluded by scarring.

Overall Total Score

Each eye was evaluated and graded by at least 2 trained corneal specialists. When the scores varied from one corneal specialist to another, the scores were averaged or determined after a discussion. The results then were added together to give an overall score from 0 to 39, with 39 representing the most severely affected eyes.

Visual Acuity

We categorized the 138 eyes from the 73 patients according to their visual acuity. In group 1 (n = 28 eyes), visual acuity was 20/20 or better, in group 2 (n = 36 eyes), it was worse than 20/20 and up to and including 20/200, in group 3 (n = 32 eyes), it was worse than 20/200 and up to and including 20/2000, and in group 4 (n = 42 eyes), it was worse than 20/2000.

Eye Complications Independent of Ocular Surface Disorders

Cataract, glaucoma, retinal diseases, or other eye diseases independent of ocular surface disorders also were evaluated and their presence, absence, or the inability to diagnosis because of ocular surface abnormalities was recorded.

Statistical Analysis

Spearman correlation coefficients (2-tailed) were used to evaluate whether the scores of the 13 components were correlated with logarithm of the minimum angle of resolution (logMAR) visual acuity. The correlation between the total score and logMAR visual acuity and the correlations between the subtotal scores of 3 problem categories and the total score also were evaluated. Using a logistic regression model, the scores for each of the 13 components

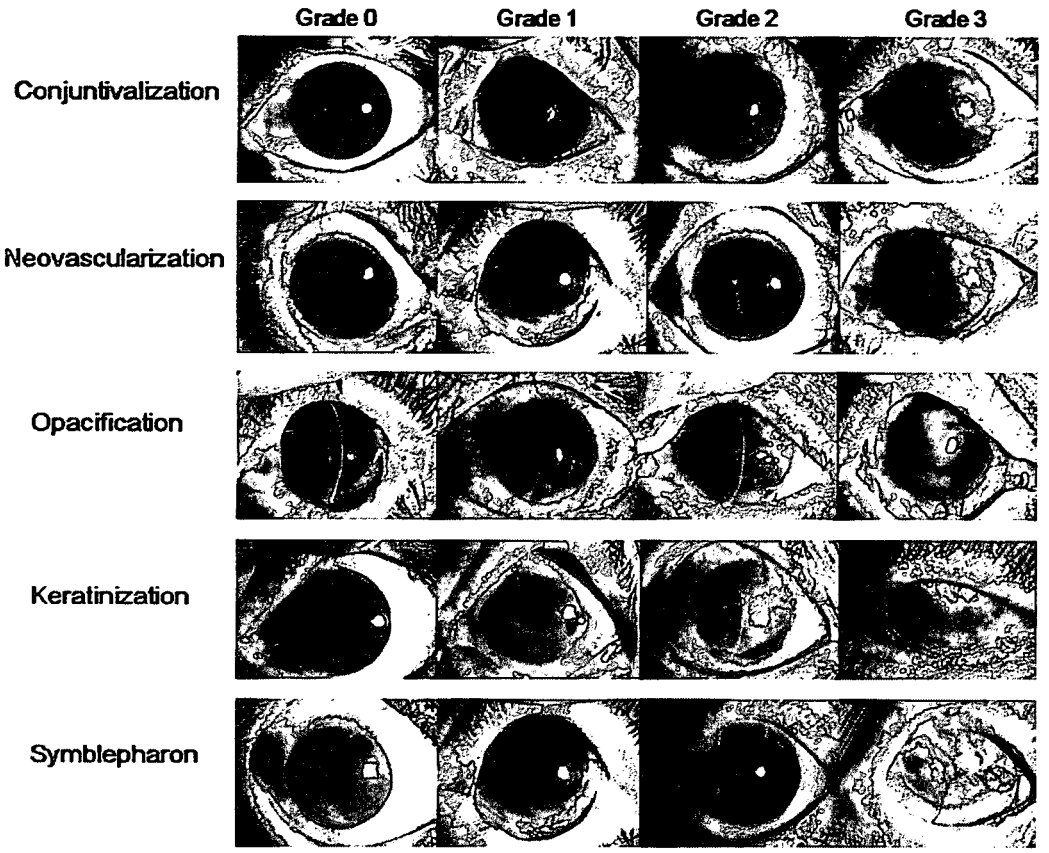

Figure 1. Grading scores of corneal and conjunctival complications.

in eyes with better visual acuity (20/200 or better; i.e., groups 1 and 2) were compared with the scores obtained for eyes with poorer visual acuity (worse than 20/200; i.e., groups 3 and 4). The statistical model for predicting logMAR visual acuity was calculated using a linear model with stepwise variable selection (multivariable regression analysis). In multivariable regression analysis, cataract and glaucoma were graded as follows: with cataract, 1; without cataract or lens invisible, 0; with glaucoma, 1; and without glaucoma or unable to diagnosis glaucoma, 0. All statistical tests were conducted at a 5% level of significance ($P = 0.05$).

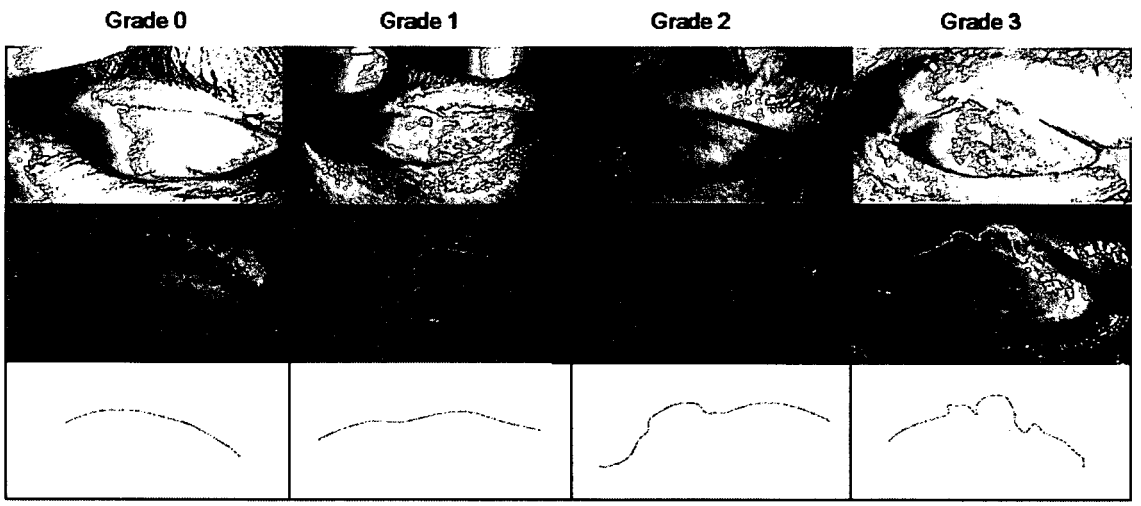

Figure 2. Grading scores of mucocutaneous junction involvement. **Top,** Grade 1 was assigned for normal mucocutaneous junction, and grades 1, 2, and 3 were assigned for mild, moderate, and severe irregularity of the mucocutaneous junction, respectively. **Middle, Bottom,** Fluorescein staining of the conjunctiva was helpful in evaluating the severity of the involvement of mucocutaneous junction.

Results

A total of 138 eyes of 73 patients from the 3 institutions were included in this study. There were 33 males and 40 females. Their age ranged from 10 to 83 years (mean±standard deviation, 47.9±18.5 years). At disease onset, the patients' ages ranged from 2 to 69 years (mean±standard deviation, 28.4±18.2 years), and the duration of the illness before seeking consultation at our centers ranged from 1 to 54 years (mean±standard deviation, 18.8±15.5 years). Drugs were the most commonly associated etiologic factor in 47 patients (64.4%). Because 14 of these patients used 2 or 3 types of drugs simultaneously, it was difficult to identify the drug(s) implicated in disease onset; therefore, we considered all their drugs to be causative. The causative drugs were antibiotics in 21 patients, cold remedies in 18 patients, nonsteroidal antiinflammatory drugs in 10 patients, anticonvulsants in 6 patients, and other in 4 patients. The precise history regarding the use of drugs was unclear in 20 patients because of the long interval between disease onset and this study.

Corneal Complications

A detailed summary of the 7 evaluated components comprising corneal complications is shown in Table 1. Among the 138 eyes examined, 114 (82.6%) manifested a total loss of POV (grade 3). Moderate to severe (grade 2 or 3) corneal SPK was present in 93 eyes (67.4%), neovascularization was present in 83 eyes (60.1%), and conjunctivalization was present in 82 eyes (59.4%).

Conjunctival and Eyelid Complications

Among the 6 evaluated components that comprise conjunctival and eyelid complications, the meibomian glands were most frequently and most severely involved; 102 of the 138 eyes (73.9%) manifested grade 3 meibomian gland involvement (Table 2). The scores for punctal damage and mucocutaneous involvement also were high; grade 2 or 3 punctal damage was assigned to 93 eyes (67.4%), and grade 2 or 3 mucocutaneous involvement was assigned to 71 eyes (51.4%).

Eye Complications Independent of Ocular Surface Disorders

Cataract was observed in 11 of 138 eyes. Glaucoma was diagnosed in 4 eyes, none of which had central loss of visual fields. There were no other eye complications independent of ocular surface disorders.

Visual Acuity

The number of eyes in each of the 4 groups was fairly evenly distributed (Table 3). Of the 138 eyes examined, 74 (53.6%) had visual acuity worse than 20/200 (group 3, n = 32; group 4, n = 42). Only 28 eyes (20.3%) had visual acuity equal to 20/20 or better.

Table 1. Summary of Corneal Complications (138 Eyes)

Complication	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Superficial punctate keratopathy	22 (15.9)	23 (16.7)	18 (13.0)	75 (54.3)
Epithelial defect	135 (97.8)	2 (1.4)	1 (0.7)	0 (0)
The loss of palisades of Vogt	21 (15.2)	3 (2.1)	0 (0)	114 (82.6)
Conjunctivalization	41 (29.7)	15 (10.9)	10 (7.2)	72 (52.2)
Neovascularization	35 (25.4)	20 (14.5)	22 (15.9)	61 (44.2)
Opacification	43 (31.2)	41 (29.7)	28 (20.3)	26 (18.8)
Keratinization	105 (76.1)	10 (7.2)	5 (3.6)	18 (13.0)

Table 2. Summary of Conjunctival and Eyelid Complications (138 Eyes)

Complications	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Conjunctival complications				
Hyperemia	46 (33.3)	61 (44.2)	15 (10.9)	16 (11.6)
Symblepharon formation	40 (29.0)	54 (39.1)	21 (15.2)	23 (16.7)
Eyelid complications				
Trichiasis	42 (30.4)	41 (29.7)	44 (31.9)	11 (8.0)
Mucocutaneous junction involvement	16 (11.6)	51 (37.0)	34 (24.6)	37 (26.8)
Meibomian gland involvement	13 (9.4)	14 (10.1)	9 (6.5)	102 (73.9)
Punctal damage	36 (26.1)	9 (6.5)	15 (10.9)	78 (56.5)

Correlation between Visual Acuity and Grade of Complications

When we compared eyes with better (20/200 or better) and worse (worse than 20/200) visual acuity with respect to the scores obtained for each of the 13 components, we found that with the exception of epithelial defect, the scores differed significantly (Table 4).

We estimated the correlation coefficient between the visual acuity of the 138 eyes and the severity grade, scored from 0 to 3, of each of the 13 evaluated components in the 3 categories of complications. We found that all 13 components were correlated significantly with logMAR visual acuity; the correlation coefficient (R) ranged from 0.359 to 0.810 ($P<0.0001$); for corneal epithelial defects, the value was $R = 0.169$ ($P = 0.0473$; Table 5). Of all the scores, corneal neovascularization, opacification, and conjunctivalization were most highly correlated with poor vision ($R = 0.810$, $P<0.0001$; $R = 0.784$, $P<0.0001$; and $R = 0.726$, $P<0.0001$, respectively).

The statistical model for predicting logMAR visual acuity was calculated using a linear model with stepwise variable selection as follows: logMAR = −0.2573 + cataract × 0.4153 + POV × 0.2814 + SPK × 0.08551 + epithelial defect × 0.3018 + neovascularization × 0.3471 + opacification × 0.3202 + keratinization × 0.1347. This multivariable regression analysis showed that corneal neovascularization, opacification, keratinization, and cataract had a significant effect on logMAR visual acuity (Table 6). The predicted logMAR was correlated significantly with the actual logMAR visual acuity measured ($R = 0.960$, $P<0.0001$).

Overall Total Score

The mean overall total score for the 13 components was 19.3±9.5 (range, 0–35). As shown in Tables 3 and 4 and Figure 3, eyes with a higher total score had poorer vision. The averaged scores for the 4 visual acuity groups were: group 1, 5.86 (range, 0–19); group 2, 16.64 (range, 2–28); group 3, 23.31 (range, 15–33); and group 4, 27.45 (range, 18–35). Pearson's analysis clearly demonstrated that the total score was significantly correlated with logMAR visual acuity ($R = 0.806$, $P<0.0001$; Fig 3). The subtotal scores of 3 problem categories correlated with the overall total score (Fig 4).

Table 3. Ocular Complications and Visual Acuity of Stevens–Johnson Syndrome Patients

Complications	Visual Acuity			
	Group 1, 20/20 or Better, Average Grade	*Group 2, 20/20 to 20/200, Average Grade*	*Group 3, 20/200 to 20/2000, Average Grade*	*Group 4, Worse than 20/2000, Average Grade*
No. of eyes	28	36	32	42
Corneal complications				
SPK	0.82	1.92	2.40	2.78
Epithelial defect	0	0	0.03	0.07
Loss of POV	0.82	2.78	3.00	3.00
Conjunctivalization	0.11	1.36	2.59	2.76
Neovascularization	0.25	1.11	2.38	2.90
Opacification	0.11	0.61	1.66	2.31
Keratinization	0.04	0.11	0.50	1.26
Conjunctival complications				
Hyperemia	0.36	0.89	1.19	1.40
Symblepharon formation	0.18	0.97	1.19	2.07
Eyelid complications				
Trichiasis	0.57	1.08	1.38	1.50
Mucocutaneous junction involvement	0.79	1.56	1.91	2.10
Meibomian gland involvement	1.32	2.50	2.69	2.90
Punctal damage	0.50	1.78	2.65	2.58
Total score	5.86	16.64	23.31	27.45

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Discussion

Severe ocular surface disease arising from SJS or TEN is associated with significant visual morbidity.[1–4] The evaluation of ocular complications in these patients is extremely important, because ocular involvement often represents the only long-term complication of SJS. There is currently no established method for evaluating the spectrum of ocular manifestations arising from these diseases. In this study, we detailed the characteristic ocular complications in the chronic stage of SJS and developed a grading system to assess more objectively the extent and severity of 13 components of these ocular complications. To the best of our knowledge, this is the first study that specifically attempted to improve and standardize the evaluation of ocular complications in SJS.

As we set out to develop a grading system that could be used easily by ophthalmologists, we identified complications that were important and could be evaluated easily by simple slit-lamp examination. After several pilot studies, we eventually settled on 13 components of 3 categories of

Table 4. Comparison between Ocular Complications and Visual Acuity

Complications	Visual Acuity of 20/200 or Better, Average Grade	Visual Acuity Worse than 20/200, Average Grade	P Value
No. of eyes	64	74	
Corneal complications			
SPK	1.44	2.62	<0.0001
Epithelial defect	0	0.05	0.1208
Loss of POV	1.92	3.00	<0.0001
Conjunctivalization	0.81	2.69	<0.0001
Neovascularization	0.73	2.68	<0.0001
Opacification	0.39	2.03	<0.0001
Keratinization	0.08	0.93	<0.0001
Conjunctival complications			
Hyperemia	0.66	1.31	<0.0001
Symblepharon formation	0.63	1.69	<0.0001
Eyelid complications			
Trichiasis	0.86	1.45	0.0002
Mucocutaneous junction involvement	1.23	2.01	<0.0001
Meibomian gland involvement	2.02	2.81	<0.0001
Punctal damage	1.26	2.61	<0.0001
Total score	11.86	25.66	

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 5. Correlation Analyses between 13 Complications and Logarithm of the Minimum Angle of Resolution Visual Acuity

Complications	Coefficient	P Value
Neovascularization	0.810	<0.0001
Opacification	0.784	<0.0001
Conjunctivalization	0.726	<0.0001
Symblepharon formation	0.649	<0.0001
SPK	0.601	<0.0001
Loss of POV	0.550	<0.0001
Punctal damage	0.518	<0.0001
Mucocutaneous junction involvement	0.488	<0.0001
Keratinization	0.477	<0.0001
Meibomian gland involvement	0.453	<0.0001
Hyperemia	0.383	<0.0001
Trichiasis	0.359	<0.0001
Epithelial defect	0.169	0.0473

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 6. Multivariable Regression Analysis

Variables	Coefficient	95% Confidence Intervals	P Values
Intercept	−0.2573	−0.5449 to 0.0303	0.0786
Neovascularization	0.3471	0.2113–0.4849	<0.0001
Opacification	0.3203	0.1734–0.4672	<0.0001
Keratinization	0.1347	0.0281–0.2413	0.0142
Cataract	0.4153	0.0249–0.8057	0.0375
Loss of POV	0.2814	−0.0784 to 0.6412	0.1228
SPK	0.0855	−0.0296 to 0.2006	0.1423
Epithelial defect	0.3018	−0.1057 to 0.7093	0.1434

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

complications that we considered important for the assessment of severe or cicatricial ocular surface disorders. We used a simple method for grading the severity of these complications; the components were assigned scores that reflected whether involvement was mild, moderate, or severe. This grading system was judged to be easy and convenient at the 3 participating ophthalmology centers that evaluated 138 eyes from 73 SJS patients. The results obtained at the 3 centers were consistent and comparable. Ours is one of few prospective studies on the ocular complications of SJS, and each patient was evaluated carefully by at least 2 ophthalmologists. To the best of our knowledge, this is the largest such study reported to date.

The initial ocular pathologic process in SJS, inflammation and necrosis of the conjunctiva, often is accompanied by the destruction of goblet cells.[4,13,14] The production of mucin by these cells is vital for maintaining an adequate tear film essential for corneal clarity. Dry eye secondary to goblet cell destruction is the most common long-term ocular complication in patients with various ocular surface diseases.[4,13,14] Cicatricial lid and conjunctival complications include symblepharon formation, forniceal shortening, keratinization, lid malposition (e.g., entropion), and misdirected eyelashes (trichiasis).[4,13,15–22] Limbal stem cell destruction, evidenced by loss of the POV, also may occur at disease onset and may be accompanied by severe inflammation. The combination of these complications may result in recurrent corneal erosion, ulceration, vascularization, stromal scarring, conjunctivalization of the corneal surface, and progressive corneal melting and perforation.[4,13,15–22]

In our study, drugs were the most commonly identified etiologic factor; in 47 patients (64.4%), antibiotics (n = 21 patients), cold remedies (n = 18 patients), or nonsteroidal antiinflammatory drugs (n = 10) were the causative agents. These findings are consistent with previous reports.[15,16,19,23]

Of all the complications, severe (grade 3) meibomian gland involvement and loss of the POV (102 and 114 eyes, respectively) were the most common ocular complications of SJS. We found that the total score for each eye was correlated significantly with its visual acuity; consistently, eyes with higher overall scores had poorer vision. We categorized the complications as those involving predominantly the cornea, the conjunctiva, and the eyelid. As expected, corneal complications were most likely to have a detrimental effect on vision. In particular, corneal neovascularization and opacification were correlated highly with posttreatment visual acuity in the chronic stage. Conjunctivalization, a sequela of limbal stem cell deficiency, also was correlated with poor vision in our series.

In our study, there was a high rate of lid complications in chronic SJS. Of the eyelid complications, meibomian gland involvement was moderate or severe (grade 2 or 3) in 111 of the 138 eyes (80.4%). We found that eyes without apparent corneal complications also manifested cicatricial eyelid changes. As such, the meibomian glands seem to be susceptible to injury after SJS. Because the meibomian glands play a critical role in the stabilization of the tear film, this is likely to contribute to the disruption of the tear film and severe dry eye condition experienced in patients in the chronic stage of SJS.

The use of a standardized method for grading the extent and severity of ocular complications in SJS patients offers

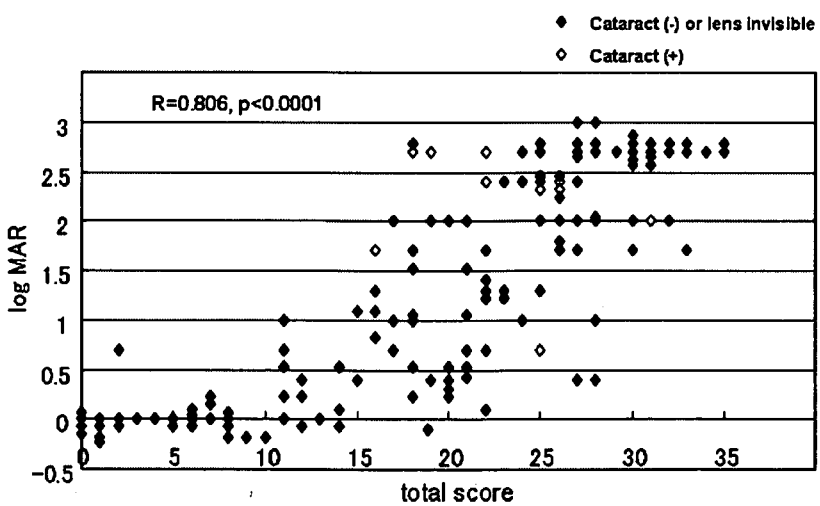

Figure 3. Scatterplot depicting the correlation between the total score and logarithm of the minimum angle of resolution (logMAR) visual acuity. The overall total score of 13 components (0–39) versus logMAR showed a significant positive correlation (Spearman $R = 0.806$, $P<0.0001$).

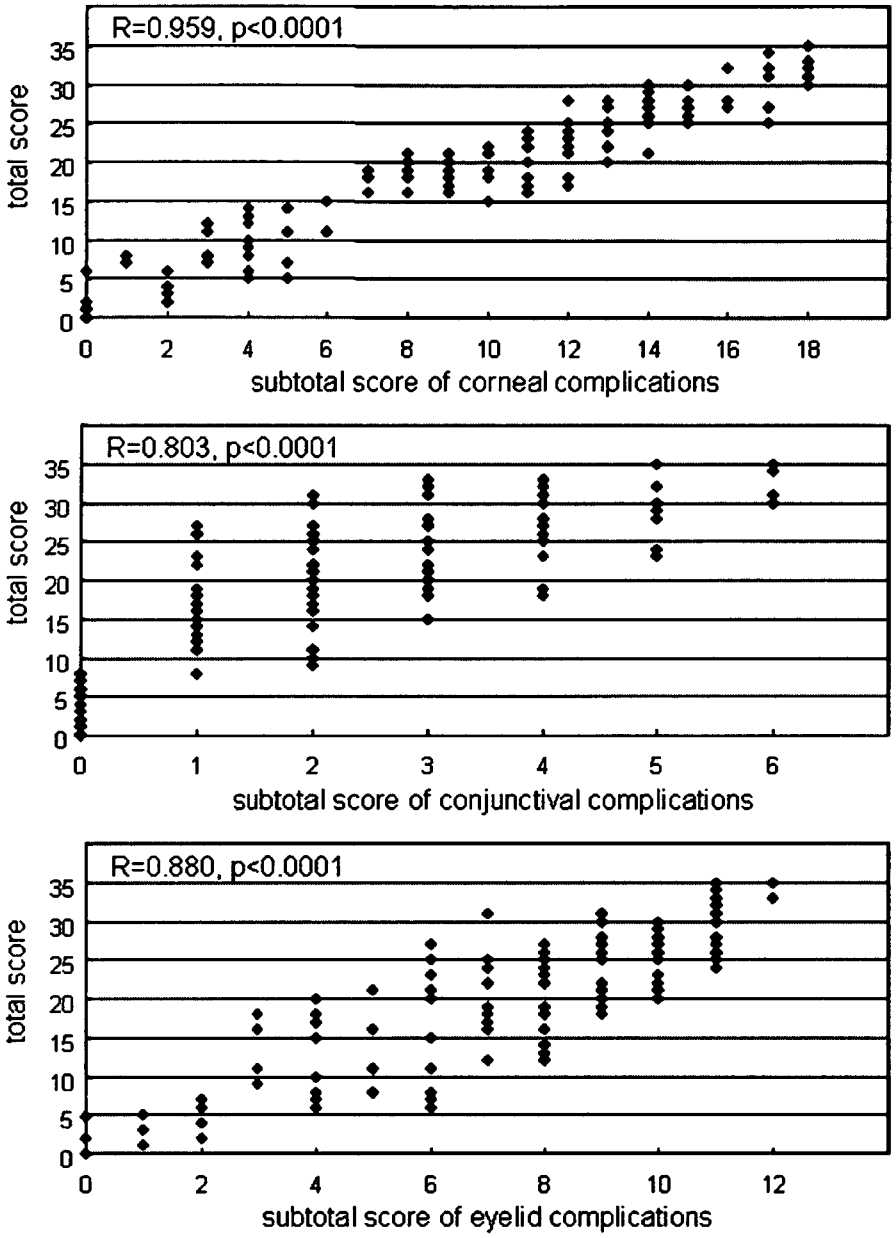

Figure 4. Scatterplots depicting the correlations between subtotal score of 3 categories and overall total score. Subtotal scores of corneal, conjunctival, and eyelid complications versus the total score all showed a significant positive correlation: (top) Spearman $R = 0.959$, $P<0.0001$; (middle) $R = 0.803$, $P<0.0001$; (bottom) $R = 0.880$, $P<0.0001$.

significant advantages. The grading system introduced here can be used in the initial evaluation and the follow-up and monitoring of ocular complications in SJS patients. As documented here, the lid margin is a commonly affected site in the disease process. However, because attention often focuses on the ocular surface, changes in the lid margin may be overlooked. Our grading system ensures that important ocular complications are detected by corneal specialists as well as nonspecialized ophthalmologists.

Ocular surface reconstructive procedures such as limbal and cultivated epithelial stem cell transplantation have been used to treat severe ocular manifestations in SJS patients.[8–11,24] However, because many of the reported studies are nonrandomized case series without control arms and because there is currently no standardized method for grading ocular complications in SJS patients in the acute and chronic stage, it is difficult to compare the treatment outcomes of these studies. Our grading system also provides a standardized method for evaluating patients before corneal and ocular surface transplantation procedures. The use of an objective method of grading the severity of the patient's preoperative condition ultimately may help in prognosticating the long-term clinical outcome of these eyes after surgery.

This is the first study that describes a method for classifying and grading the severity of ocular involvement in SJS patients. Our findings have important clinical implications and facilitate the objective evaluation of patients with ocular complications resulting from SJS. The method presented here may be adapted for use in patients with cicatricial ocular surface diseases arising from other causes such as ocular cicatricial pemphigoid and chemical injury. It also provides a common platform for the discussion and management of patients with ocular surface disorders and may be useful for predicting treatment outcomes. Our method also enables ophthalmologists to monitor more objectively the progression of complications during the follow-up of these patients.

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EXTENDED REPORT

Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens–Johnson syndrome

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Background and aim: Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute-onset mucocutaneous diseases induced by infectious agents and/or inciting drugs. Given the association between the onset of SJS/TEN and infections, the possibility that there is an association between SJS/TEN and a disordered innate immune response was considered. The first line of defence against infection is comprised of evolutionarily conserved sets of molecules, the Toll-like receptors (TLRs). TLR3 recognises double-stranded RNA associated with viral infections.

Methods: The Japanese single-nucleotide-polymorphism (JSNP) database reports 7 polymorphisms consisting of 7 SNPs in the human TLR3 gene; 3 of the 7 SNPs are coded in exon regions, (ie, 293248A/G, 293391A/G and 299698T/G), and the other 4 are coded in intron regions, (ie, 294440G/C, 294732C/T, 208036T/C and 298054C/T). These 7 SNPs were analysed in 57 Japanese patients with SJS/TEN with ocular surface complications and in 160 Japanese healthy controls.

Results: SNP 299698T/G and the genotype patterns of 293248A/A and 299698T/T were strongly associated with SJS/TEN.

Conclusion: The results suggest that polymorphisms in the TLR3 gene could be associated with SJS/TEN in the Japanese population.

Stevens–Johnson syndrome (SJS) and the related disease toxic epidermal necrolysis (TEN) are acute multisystem inflammatory disorders of the skin and mucous membranes, including the ocular surface. They are commonly associated with infectious agents and/or an inciting drug.[1] The annual incidence of SJS and TEN has been estimated as 0.4–1 and 1–6 cases per million persons, respectively,[1 2] and the mortality is 3% and 27%, respectively.[3] Although SJS and TEN are rare, they carry high morbidity and mortality, and often result in severe handicaps such as loss of vision. The rarity of cutaneous, mucosal and ocular surface reactions to drug therapies led us to suspect individual susceptibility. Therefore, we examined the possibility of a genetic predisposition towards SJS/TEN.

SJS/TEN is one of the most devastating ocular surface diseases, leading to corneal damage and loss of vision. The reported incidence of ocular complications in SJS/TEN is 50–68%.[1 3] Ophthalmologically, in the acute stage, patients with SJS/TEN manifest vesiculobullous skin lesions, severe conjunctivitis and persistent corneal epithelial defects because of ocular surface inflammation. In the chronic stage, ocular surface complications such as conjunctival invasion into the cornea due to corneal epithelial stem-cell deficiency, symblepharon and ankyloblepharon, and in some instances keratinisation of the ocular surface, persist (fig 1A) despite healing of the skin lesions.[4] We observed that >95% of the patients with SJS/TEN with ocular surface complications had lost their finger nails in the acute or subacute stage, and that some continue to have transformed nails even after healing of the skin lesions (fig 1B). In the current study, we focused exclusively on patients with SJS/TEN accompanied by ocular surface complications.

Drugs are probably the most widely accepted aetiological factor in SJS/TEN.[5] In addition, patients with SJS/TEN often had prodromata, including non-specific fever, coryza and sore throat, that closely resemble upper respiratory tract infections commonly treated with antibiotics. These prodromata were evident from the clinical records of our patients with SJS/TEN. *Mycoplasma pneumoniae* was responsible for 5 of 17 cases of childhood SJS,[6] and a viral aetiology involving herpes simplex, Epstein–Barr, cytomegalo-, and varicella zoster virus has been reported.[7 8]

Given the association between the onset of SJS/TEN and infections, and the opportunistic infection of ocular surfaces by bacteria such as methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis*,[9] we considered the possibility of an association between SJS/TEN and a disordered innate immune response. We postulated that viral infection and/or drugs might trigger a disorder in the host's innate immune response, and that this event is followed by aggravated inflammation of the mucous membranes, ocular surface and skin.

The first line of defence against infection is comprised of evolutionarily conserved sets of molecules, the Toll-like receptors (TLRs). The triggering of TLRs results in the secretion of antibacterial peptides and proinflammatory cytokines. The inflammatory response results in the recruitment of cells of adaptive immunity to initiate clearance of the pathogens. TLR3 recognises double-stranded (ds)RNA associated with viral infections, a component of the life cycle of most viruses.[10] As functional deterioration of TLR3 can predispose individuals to increased susceptibility to viral infections, the detection of TLR3 polymorphisms could yield critical information for risk assessment regarding susceptibility to microbial infections in the context of SJS/TEN.

To date, no reports are available on the genetic loci of TLR3 in subjects with SJS/TEN. Therefore, we performed single-nucleotide polymorphism (SNP) association analysis of the

Abbreviations: ds RNAs, double stranded RNAs; RV, rhinovirus; SJS, Stevens–Johnson syndrome; SNP, single-nucleotide polymorphism; TEN, toxic epidermal necrolysis; TLR3, Toll-like receptor 3

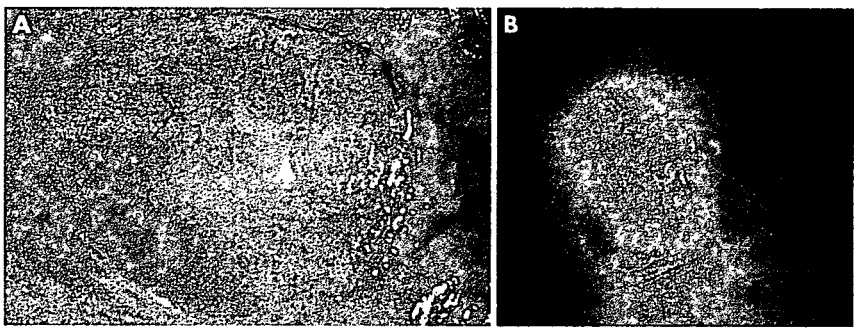

Figure 1 Patients with Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) with ocular complications. (A) Ocular surface complications such as conjunctival invasion into the cornea, symblepharon and ankyloblepharon, and sometimes keratinisation of the ocular surface, persist in some patients with SJS/TEN in the chronic stage. Conjunctival invasion results in severe vision loss. (B) Transformed fingernails of patients with SJS/TEN with ocular complications. Many patients with SJS/TEN with ocular complications lost their fingernails during the acute stage, and some continue to have transformed nails even after healing of the skin lesions. The photograph shows the thumbnail of a 26-year-old man 6 years after onset of the disease (chronic stage).

TLR3 gene, which maps to chromosome 4q35. The Japanese single-nucleotide polymorphism (JSNP) database reports seven polymorphisms consisting of seven SNPs in the human TLR3 gene: three of the seven SNPs are coded in exon regions ie, 293248A/G (rs.3775290, exon 4, silent SNP), 293391A/G (rs.3775291, exon 4, change SNP) and 299698T/G (rs.3775296, exon 2, UTR SNP), and the other four are coded in intron regions (ie, 294440G/C (rs.3775292, intron 3), 294732C/T (rs.3775293, intron 3), 208036T/C (rs.3775294, intron 2) and 298054C/T (rs.3775295, intron 2; fig 2).

We analysed these seven SNPs in 57 Japanese patients with SJS/TEN with ocular surface complications and in 160 healthy Japanese controls. We found that SNP 299698T/G and the genotype patterns of 293248A/A and 299698T/T are strongly associated with SJS/TEN.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review board of the Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Declaration of Helsinki. The purpose of the research and the experimental protocols were explained to all participants, and their prior written informed consent was obtained.

For SNP analysis, we enrolled 57 patients with SJS/TEN in the chronic or subacute phase; all presented with ocular surface complications. The diagnosis of SJS/TEN was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions and involvement of at least two mucosal sites, including the ocular surface. The controls were 160 healthy volunteers. All participants and volunteers were Japanese, residing in Japan. The mean (SD) age of the patients involved in this study was 45.2 (17.5) years, and that of the controls was 36.2 (11.5) years. The ratios of men/women in the patient and control groups were 24/33 and 57/103, respectively.

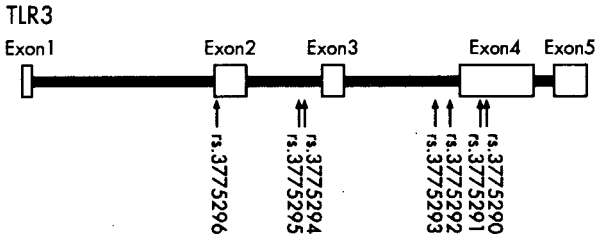

Figure 2 Genomic organisation of the Toll-like receptor 3 (TLR3) gene on chromosome 4q35. The genomic organisation of the gene was derived from the Japanese single-nucleotide polymorphism (JSNP) database. Seven polymorphisms consisting of seven SNPs have been reported in the human TLR3 gene in the JSNP database; three of the seven SNPs are coded in exon regions (ie, 293248A/G (rs.3775290, exon 4, silent SNP), 293391A/G (rs.3775291, exon 4, change SNP) and 299698T/G (rs.3775296, exon 2, UTR SNP)), and the other four are coded in intron regions (ie, 294440G/C (rs.3775292, intron 3), 294732C/T (rs.3775293, intron 3), 208036T/C (rs.3775294, intron 2) and 298054C/T (rs.3775295, intron 2)).

SNP analysis

TLR3 SNP analysis was performed by sequencing from both sides, forward and reverse, to confirm the results carefully. For SNPs of TLR3, the PCR and sequence primers were 5'-TGGCTAAAATGTTTGGAGCA-3' (sense) and 5'-GAAGAGGCTGGAATGGTGAA -3' (antisense) for rs.3775290 and rs.3775291, 5'-CAGTTCTTTACTCCATCTCCGC-3' (sense) and 5'-CCAAGGCTCTGGTAAGGGTG-3' (antisense) for rs.3775292 and rs.3775293, 5'-TCACATGGCTTATCAAACACACAG-3' (sense) and 5'-CATTGCTCTTCCTCAGATGCC-3' (antisense) for rs.3775294 and rs.3775295, and 5'-TTACCTTCTGCTTGACAAAGGG-3' (sense) and 5'-TGCATTTGAAAGCCATCTGC-3' (antisense) for rs.3775296. All primers except for rs.3775290 and rs.3775291, were those recommended in the JSNP database. Genomic DNA was isolated from human peripheral blood at SRL (Tokyo, Japan). PCR amplification was done with DNA polymerase (Takara; Shiga, Japan) for 35 cycles at 94℃ for 1 min, and annealing at 60℃ for 1 min and at 72℃ for 1 min on a commercial PCR machine (GeneAmp; Perkin-Elmer Applied Biosystems, Foster City, California, USA). The PCR products were reacted with BigDye Terminator V.3.1 (Applied Biosystems, Foster City, California, USA), and sequence reactions were resolved on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Statistical methods

Alleles as well as genotype patterns were counted manually. For Hardy–Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions, we used the χ^2 test. The odds ratio (OR) with 95% CI was calculated using Labo Server software (World Fusion, Tokyo, Japan). Each allele and genotype pattern was assessed as an independent variable, and separate p values were calculated for each polymorphism. A p value of <0.05 was regarded as significant. In addition, the p values were corrected for the number of alleles tested (Bonferroni method).

RESULTS AND DISCUSSION

Table 1 shows a summary of our case–control association study on the seven SNPs genotyped to TLR3.

All but SNP 294732C/T were in Hardy–Weinberg equilibrium in both patients with SJS/TEN and controls ($p>0.05$). SNP 299698T/G showed a significant association under a recessive model (299698 T/G + G/G vs T/T, raw p value = 0.001, corrected

Table 1 Genotype frequencies of toll-like receptor 3 single-nucleotide polymorphisms among Japanese patients with Stevens-Johnson syndrome/ toxic epidermal necrolysis and healthy controls

	Controls n (%) (n = 160)	SJS/TEN n (%) (n = 57)	Allele 1 vs. Allele 2 value(χ^2) OR (95% CI)	Genotype 11 vs 12+22 value(χ^2) OR (95% CI)	Genotype 11+12 vs 22 value(χ^2) OR (95% CI)
293248 (rs.3775290)					
11 GG	63 (39.4)	19 (33.3)	0.110	0.419	0.046
12 GA	78 (48.8)	25 (43.9)	–	–	0.456
22 AA	19 (11.9)	13 (22.8)	(–)	(–)	(0.209 to 0.997)
93391 (rs.3775291)					
11 GG	84 (52.5)	34 (59.7)	0.271	0.352	0.400
12 GA	62 (38.8)	20 (35.1)	–	–	–
22 AA	14 (8.8)	3 (5.3)	(–)	(–)	(–)
294440 (rs.3775292)					
11 CC	149(93.1)	51 (89.5)	0.388	0.378	–
12 CG	11 (6.9)	6 (10.5)	–	–	–
22 GG	0 (0)	0 (0)	(–)	(–)	
294732 (rs.3775293)					
11 TT	160(100)	56 (98.2)	–	–	–
12 TC	0 (0)	1 (1.8)	–	–	–
22 CC	0 (0)	0 (0)			
298036 (rs.3775294)					
11 CC	145(90.6)	49 (86.0)	0.340	0.326	–
12 CT	15 (9.4)	8 (14.0)	–	–	–
22 TT	0 (0)	0 (0)	(–)	(–)	
298054 (rs.3775295)					
11 TT	54 (33.8)	23 (40.4)	0.541	0.388	0.974
12 TC	75 (46.9)	23 (40.4)	–	–	–
22 CC	31 (19.4)	11 (19.3)	(–)	(–)	(–)
299698 (rs.3775296)					
11 GG	77 (48.1)	26 (45.6)	0.095	0.744	0.001
12 GT	75 (46.9)	20 (35.1)	–	–	0.220
22 TT	8 (5.0)	11 (19.3)	(–)	(–)	(0.084–0.580)

SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

p value = 0.007, OR = 0.22). However, when we corrected the p value for the number of alleles tested (n = 7), the results were no longer significant; SNP 293248A/G also showed a significant association under a recessive model (293248 A/G + G/G vs A/A, raw p value = 0.046, corrected p value = 0.322, OR = 0.46).

We also analysed the genotype patterns of SNPs 299698T/G and 293248A/G and found that these (293248A/A–299698T/T) were also strongly associated with SJS/TEN in Japanese patients (χ^2 test, p = 0.001, OR = 5.5, 95% CI = 1.9 to 15.8) (table 2). This association was stronger than that observed for the single locus (299698T/G).

Our results suggest that polymorphisms in the TLR3 gene could be associated with SJS/TEN in the Japanese population. We hypothesised that viral infection and/or drugs might trigger a disorder in the host innate immune response, and that this event is followed by aggravated inflammation of the mucous membranes, ocular surface and skin. Genetic and environmental factors could play a role in an integrated aetiology of SJS/TEN.

Because the 299698T/G SNP, which showed a significant association with SJS/TEN, is encoded in the exon region, we consider it important to extend this study by performing expression and function analysis of the TLR3 protein with this SNP. According to the International HapMap project, the 299698T/G (rs.3775296) SNP exists not only in Japanese (G/G 0.386, G/T 0.500, T/T 0.114) but also in Han Chinese (G/G 0.659, G/T 0.295, T/T 0.046) and Caucasian (G/G 0.719, G/T 0.263, T/T 0.018) populations, indicating that it is important to examine TLR3 SNPs in non-Japanese populations.

TLR3 is involved in responses to dsRNAs.[10] As rhinoviruses are a major cause of the common cold and the acute exacerbation of chronic obstructive pulmonary disease, the functional requirement for TLR3 in the host response against infection with live viruses, especially rhinovirus infection, has been proposed.[11]

The association documented here complements previous findings compatible with an unregulated innate immune response as an important pathophysiological condition in inflammatory ocular surface diseases.[12–14] SJS/TEN could be the consequence of exposure of genetically susceptible individuals to specific environmental precipitants. A report from the US showed that the HLA (human leucocyte antigen)-B12 HLA-Bw44 antigen was significantly increased in Caucasian patients with SJS with ocular involvement.[15] Analyses of patients with TEN in France also disclosed an association with HLA-B12 (HLA-Bw44).[16] In Han Chinese, there was a very strong association between carbamazepine-induced SJS and the HLA-B*1502 allele.[17] Elsewhere we reported that, in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN, with ocular surface complications.[18] These findings suggest that SJS/TEN is associated with a complex genetic-inheritance background, and that specific combinations of genes are required for disease-onset.

The pathophysiological mechanisms underlying the onset of SJS/TEN have not been fully established, although the involvement of immune mechanisms and altered drug metabolism has been suggested.[19–23] Heretofore, it was not recognised that innate immunity plays a critical role in the pathophysiology of SJS/TEN, by way of bridging between the acute response to invading non-self molecules and chronic local immune inflammation.

We previously reported that while human corneal epithelium harbours messages for most TLRs, TLR3 is most highly expressed.[12] In conjunctival, as in corneal, epithelium, TLR3 is the TLR with the highest expression level at the messenger RNA

Table 2 Pattern structures and frequency of single-nucleotide polymorphisms 293248A/G and 299698T/G

Pattern type	293248 A/G	299698 T/G	Control n (%) (n = 160)	SJS/TEN n (%) (n = 57)	p Value (χ^2)	OR (95% CI)
1	G/G	G/G	57 (35.6)	18 (31.6)	0.5813	-
2	A/G	T/G	57 (35.6)	16 (28.1)	0.2999	-
3	A/G	G/G	18 (11.3)	8 (14.0)	0.5782	-
4	A/A	T/G	12 (7.5)	3 (5.3)	0.5675	-
5	G/G	T/G	7 (4.4)	1 (1.8)	0.3673	-
6	A/A	T/T	6 (3.8)	10 (17.5)	0.0006	5.5 (1.9 to 15.8)
7	A/G	T/T	2 (1.3)	1 (1.8)	0.7794	-
8	A/A	G/G	1 (0.6)	0 (0)	0.5497	-

SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

level (data not shown). We reported that the cell-surface TLR3 of human corneal epithelial cells responds to virus dsRNA-mimic polyI:C to generate proinflammatory cytokines and interferon β, and that the innate immune responses in human corneal epithelial cells differ from those in immune-competent cells.[12] In the current study, we clarified the association between Japanese patients with SJS/TEN and TLR3 gene polymorphisms. This raises the possibility that abnormalities of TLR3 on the ocular surface might contribute to ocular surface inflammation such as SJS/TEN. In addition, the association between the onset of SJS/TEN and viral infections raises the possibility of an association between SJS/TEN and a disordered innate immune response.

The association between TLR polymorphisms and human diseases has been suggested. Polymorphisms in the TLR3 gene could be associated with type 1 diabetes in black people from South Africa.[24] In the children of European farmers, there was a strong association between TLR2 polymorphisms and allergic diseases.[25] Torok *et al*[26] reported an association between a functional polymorphism in TLR4 and ulcerative colitis. The specific link between exposure to environmental triggers and the induction of a highly restricted autoimmune process remains to be detected, and the innate immune system could constitute a link between the environment and the adaptive immune system.

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Competing interests: None declared.

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Association of *IL4R* polymorphisms with Stevens-Johnson syndrome

To the Editor:

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute-onset mucocutaneous diseases induced by infectious agents and/or inciting drugs.[1]

Although the pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established, the extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility. In the acute stage, patients with SJS/TEN manifest vesiculobullous skin lesions, severe conjunctivitis, and persistent corneal epithelial defects. In the chronic stage, ocular surface complications such as conjunctival invasion into the cornea due to corneal epithelial stem cell deficiency persist (Fig 1, *A*). We observed that more than 95% of patients with SJS/TEN with ocular surface complications had lost their fingernails in the acute or subacute stage and that some continue to have transformed nails even after healing of the skin lesions (Fig 1, *B*). Our study focused on patients with SJS/TEN accompanied by ocular surface complications.

Previous reports suggested that SJS might have an allergic basis with drugs and infectious agents and that allergic predisposition may play a role in the development of SJS.[1] We performed polymorphism analysis of the allergy-related gene *IL4R* to examine the association between SJS/TEN and allergic predisposition.

In this study, we analyzed polymorphisms of Ile50Val (rs.1805010), Ser478Pro (rs.1805015), and Gln551Arg (rs.1801275) in the *IL4R* gene between Japanese patients with SJS/TEN and Japanese healthy volunteers. We also examined the serum IgE levels.

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Helsinki Declaration. For single nucleotide polymorphism (SNP) analysis, we enrolled 70 patients with SJS/TEN; all presented with ocular surface complications. The controls were 160 healthy volunteers. All participants and volunteers were Japanese residing in Japan. The average age of the patients and controls was 45.8 ± 17.6 (SD) and 36.2 ± 11.5 (SD) years, respectively. The male:female ratio in the patient and control groups were 31:39 and 57:103, respectively. SNP analysis was performed by direct sequencing.

For total IgE measurement and antigen-specific IgE assay, we enrolled 30 patients with SJS/TEN in the chronic phase. The controls were 160 and 30 healthy volunteers, respectively. Antigen-specific IgE were detected at SRL Inc (Tokyo, Japan) by using MAST-26 (Multiple Antigen Simultaneous Test; Hitachi, Tokyo, Japan), an allergy testing system.

A summary of the case-control association study with the 3 genotyped SNPs is shown in Table I. All 3 SNPs were in Hardy-Weinberg equilibrium in both the patients with SJS/TEN and healthy controls ($P > .01$). Among the 3 SNPs of *IL4R*, Gln551Arg showed a significant association with allele frequency (A vs G, raw P value = .0019; corrected P value = .0057; odds ratio, 3.4) and the dominant model (A/A vs A/G + G/G, raw P value = .0025; corrected P value = 0.0075; odds ratio, 3.5). SJS/TEN was associated with Gln551Arg, shown to have no effect on IgE synthesis,[2] but not with Ile50Val and Ser478Pro, which are associated with IgE synthesis.[2,3] Furthermore, in Gln551Arg polymorphisms, although Arg551 alleles were significantly increased in atopy[4] and asthma,[5] in patients with SJS/TEN, Gln551- but not Arg551 alleles were significantly increased. Next we investigated the relationship between serum IgE and SJS/TEN; we assayed both total and antigen-specific IgE. As shown in Table II, there was no significant difference between patients with SJS/TEN and the controls with respect to the incidence of high total serum IgE. In addition, assessment of positivity for antigen-specific IgE showed no marked difference between the 2 groups (Table III). Our results indicate that serum IgE was not associated with SJS/TEN and coincide with the finding that it was associated with Gln551Arg, which has no effect on IgE synthesis.

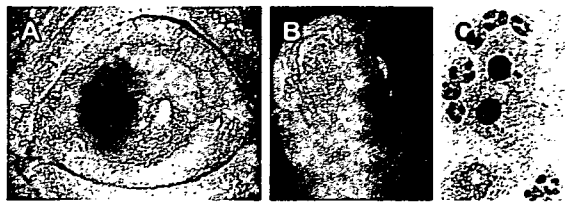

FIG 1. A, Ocular surface complications in patients with SJS/TEN in the chronic stage. **B,** Transformed fingernail in thumb of a 7-year-old girl, 5 months from SJS/TEN onset (subacute stage). Note the persistent nail bed inflammation. **C,** Discharge from the conjunctival sac of patients with SJS/TEN in the subacute stage.

A strong genetic predisposition underlies the manifestation of allergic diseases.[2-5] *IL4R* is representative of the candidate genes for atopy and asthma, which are biologically linked to T_H2 cytokine–driven inflammatory mechanisms.[2-5]

Our results show that in Japanese patients with SJS/TEN, there might be an association with polymorphisms in the *IL4R* gene. However, on the basis of our findings, we posit that SJS/TEN is different from allergic diseases such as atopy and asthma because the ratio of each allele of the polymorphisms was opposite that reported in atopy and asthma.

It has been suggested that the pathogenesis of TEN involves cytotoxic $CD8^+$ lymphocytes.[6] Because $CD8^+$ T cells involve T_H1 cytokine–driven inflammatory mechanisms, such mechanisms may be involved in the skin inflammation seen in the acute stage of SJS/TEN. In contrast, T_H2 cytokine–driven inflammatory mechanisms