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REPORTING VISUAL ACUITIES

The AJO encourages authors to report the visual acuity in the manuscript using the same nomenclature that was used in gathering the data provided they were recorded in one of the methods listed here. This table of equivalent visual acuities is provided to the readers as an aid to interpret visual acuity findings in familiar units.

Table of Equivalent Visual Acuity Measurements

Snellen Visual Acuities				
4 Meters	6 Meters	20 feet	Decimal Fraction	LogMar
4/40	6/60	20/200	0.10	+1.0
4/32	6/48	20/160	0.125	+0.9
4/25	6/38	20/125	0.16	+0.8
4/20	6/30	20/100	0.20	+0.7
4/16	6/24	20/80	0.25	+0.6
4/12.6	6/20	20/63	0.32	+0.5
4/10	6/15	20/50	0.40	+0.4
4/8	6/12	20/40	0.50	+0.3
4/6.3	6/10	20/32	0.63	+0.2
4/5	6/7.5	20/25	0.80	+0.1
4/4	6/6	20/20	1.00	0.0
4/3.2	6/5	20/16	1.25	−0.1
4/2.5	6/3.75	20/12.5	1.60	−0.3
4/2	6/3	20/10	2.00	−0.3

From Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982;94:91–96.

Functional Visual Acuity in Stevens-Johnson Syndrome

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• PURPOSE: To evaluate the correlation of functional visual acuity (FVA) measurement with ocular surface findings in patients with Stevens-Johnson syndrome (SJS).
• DESIGN: Prospective comparative study.
• METHODS: Sixty-nine eyes of 38 patients with chronic SJS assessed at the Tokyo Dental College, Tokyo Medical Center, and the Kyoto Prefectural University of Medicine, Department of Ophthalmology, Kyoto, Japan, were studied. Twenty eyes of 10 normal subjects and 40 eyes of 20 patients with Sjögren syndrome (SS) were also studied. Conventional Landolt visual acuity (VA) and FVA examinations and slit-lamp examinations were performed. FVA was measured continuously by the FVA measurement system during a 30-second blink-free period in one eye. The visual maintenance ratio (VMR) was calculated as follows: VMR = [(2.7 − FVA)/(2.7 − baseline VA)], where logarithm of minimal angle of resolution values of FVA were entered into the formula and 2.7 represented the lowest visual acuity in this series. Slit-lamp examinations, Schirmer test, and fluorescein vital stainings were also performed in all subjects.
• RESULTS: VMR was markedly lower in patients with SJS compared with patients with SS and controls. FVA values showed a relation with the presence of corneal opacity and vascularization.
• CONCLUSIONS: The FVA measurement system is not only a useful tool in the evaluation of dynamic VA changes, but also reflects the ocular surface clinical findings in SJS. (Am J Ophthalmol 2006;142: 917–922. © 2006 by Elsevier Inc. All rights reserved.)

Accepted for publication Jul 27, 2006.

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STEVENS-JOHNSON SYNDROME (SJS) IS A SEVERE OCULAR surface disease that may be associated with poor visual prognosis.[1–3] Symblepharon, adhesive occlusion of the lacrimal puncta, and corneal opacification with conjunctivalization are often observed in the chronic stages of the disease.[4] Severe dry eye due to the absence of reflex tearing is another major problem in such patients,[5] leading to worsening of ocular-surface health.[2,6,7] It has been reported that a stable tear film over the corneal surface is essential for clear visual imaging and that irregular corneal surface resulting from dry eyes is associated with a poor quality of vision.[8–12]

The functional visual acuity (FVA) measurement system is thought to reflect the dynamic changes in visual acuity (VA) reflecting the status of vision related to daily activities. With the FVA measurement system, dynamic visual changes are continuously measured under a 30-second blink-free period in one eye. We previously reported that FVA was worse in patients with Sjögren syndrome (SS) and non-SS dry eyes compared with controls.[13] In this report, we performed FVA measurements in patients with SJS and compared the results with normal, healthy subjects and in patients with SS. We also studied the correlation between FVA measurements and the clinical findings of the ocular surface status in patients with SJS.

METHODS

SIXTY-NINE EYES OF 38 CONSECUTIVE PATIENTS (20 MALE and 18 female patients; mean ± SD age 42.6 ± 15.4 years; range, eight to 74 years) with SJS assessed at the Cornea Subspecialty Outpatient Clinic of the Department of Ophthalmology of Tokyo Dental College, Tokyo Medical Center, and Kyoto Prefectual University, Japan, were studied. This research followed the tenets of the Declaration of Helsinki. Informed content was obtained from all subjects. Ethic committee approvals were obtained at each center for this study.

The diagnosis of SJS was based on the history of the presence of cryptogenic fever and acute inflammation of

0002-9394/06/$32.00
doi:10.1016/j.ajo.2006.07.055

TABLE 1. Grading of Severity Scores of Ocular Surface Clinical Findings in Patients With Stevens-Johnson Syndrome and Sjögren Syndrome

Finding	Score			
	0	1	2	3
Trichiasis	None	<1/4 of upper and lower lid	≥1/4, <1/2 of upper and lower lid	≥1/2 of upper and lower lid
Symblepharon	None	Within conjunctival sac	<1/2 of cornea	≥1/2 of cornea
MGD				
Secretion	Meibum clear	Yellow cloudy	Granular, cheesy	Not expressible
MCJ	Normal	<1 mm behind NMCJ	≥1, <2 mm behind NMCJ	≥2 mm behind NMCJ
Conjunctivalization	None	<1/4 of cornea	≥1/4, <1/2 of cornea	≥1/2 of cornea
Central corneal opacity	None	Pupillary area visible	Lens hardly visible	Pupillary area not visible
Corneal vessels	None	Peripheral	At pupillary edge	Over the pupil
Fluorescein staining (points)	0	1~2	3~5	6~9

MGD = meibomian gland dysfunction; MCJ = mucocutaneous junction; NMCJ = normal mucocutaneous junction.

mucosal membranes after receiving antibiotic or anti-inflammatory drugs, and on the presence of the chronic ocular surface complications such as symblepharon, entropion, trichiasis, xerophthalmia, and corneal vascularization.[13–16] Twenty eyes of 10 healthy normal subjects (two male and eight female subjects; mean age, 42.5 ± 12.9 years; range, 25 to 70 years) without dry eyes and dry eye symptoms and 40 eyes of 20 patients with SS (two male and 18 female subjects; mean ± SD age 52.6 ± 15.4 years; range, 19 to 80 years) who were diagnosed according to Fox criteria were also investigated in this multicenter study.[17] None of the patients or control subjects had any other systemic or ocular diseases, history of ocular surgery within six months, or history of ocular cicatricial pemphigoid, chemical, thermal, or radiation injury that would have adverse ocular surface effects. Patients with SJS with a baseline conventional best-corrected Landolt VA score of less than 20/2000 as a result of cataract, ocular surface keratinization, glaucoma, or posterior segment disease were excluded from this study.

All study subjects underwent slit-lamp examinations including assessment of the status of eyelids, presence of meibomian gland disease, conjunctivalization of the cornea, presence of corneal opacity, and assessment of corneal vessels. A severity grade was assigned for each clinical finding (Table 1). The grading of Bron and associates[18] was used for the classification of mucocutaneous junction and meibum changes. The grading and ocular surface tests were performed under the same protocol discussed and accepted before the initiation of the study by the researchers of all contributing study centers.

The standard Schirmer test without topical anesthesia was performed. The standardized strips of filter paper (Alcon Inc, Fort Worth, Texas, USA) were placed in the lateral canthus away from the cornea and left in place for five minutes with the eyes closed. Readings were reported in millimeters of wetting for five minutes. A 2-μl volume of fluorescein dye 1% was instilled in the conjunctival sac by a micropipette. The minimum score for corneal fluorescein staining was zero points and the maximum score was nine points.[19] A vital staining severity grading was also assigned in this study (Table 1).

The FVA measurement system (Nidek, Tokyo, Japan) was used to examine the timewise change in continuous VA (Figure 1). The device is made up of three parts: a hard disk, a monitor, and a joystick. The Landolt optotypes are presented on the monitor, and their sizes change depend-

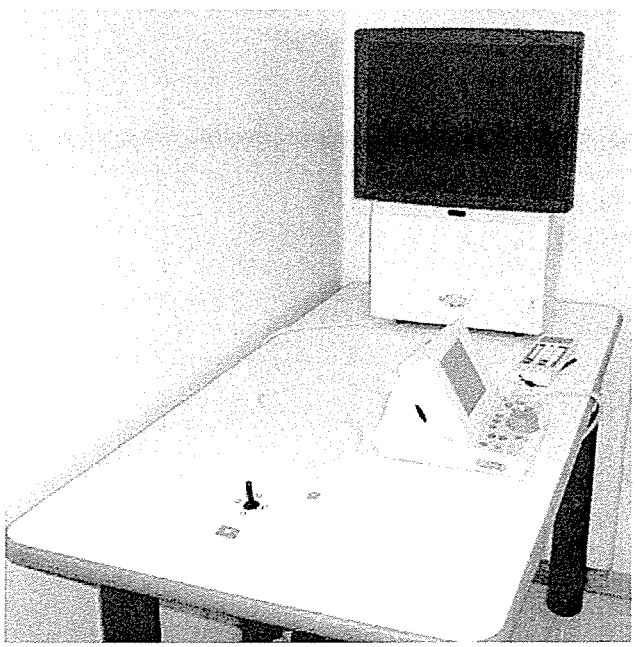

FIGURE 1. Functional visual acuity (VA) measurement system, which measures dynamic change in VA.

ing on the correctness of the responses. In brief, the optotypes are displayed automatically, starting with smaller ones. If the responses are incorrect, larger optotypes are presented automatically. When there was no response within the set display times, the answer was taken to be an error and the optotype automatically enlarged.

VA is continuously measured from the baseline best-corrected Landolt VA. The FVA measurement system can measure VA from 400/200 to 20/2000. The measurements were begun from the baseline established best-corrected Landolt conventional VA in each subject. Optotypes were presented at a distance of 2.5 m in patients with a baseline best-corrected VA ≥20/200. In subjects with a best-corrected VA <20/200, the testing distance was set at 1 m. The testing distance information is entered into the computer database so that each Landolt optotype presented on the monitor subtended an equivalent angle to the optotype of the same VA level presented from 5 m during the conventional Landolt VA testing. The presentation time of an optotype was adjusted at one second, and optotypes changed automatically within the previously set presentation time frame.

At the FVA measurement, one drop of topical anesthesia (oxybuprocaine chloride 0.4%) was administered 15 minutes before the examination to minimize discomfort and prevent reflex tearing and blinking. The upper eyelids of patients with SJS were elevated gently to allow continuous testing for 30 seconds. Patients delineated the orientation of the automatically presented Landolt rings by handling the joystick from the baseline best-corrected VA from the start. VA at 10, 20, and 30 seconds were checked as records of FVA and compared between patients with SJS, patients with SS, and control subjects. The FVA measurement system reported in this study is a new advanced version compared with the system previously reported by us, which allowed testing at 1.1 m only where the presented optotypes within the system subtended equivalent angles to the optotypes of the same VA level presented from 5 m. The previous device could not measure VA scores less than 8/200 and was also designed for clinical settings without enough distance/space to carry out vision measurements from 5 m. The current system not only allows functional VA testing at different distances, but also assesses VA in low-vision patients with VA scores ≥20/2000.

The baseline conventional best Landolt visual acuities, which were also the starting point for FVA measurements, were 20/20 or above in all patients with SS and normal control subjects. The baseline conventional Landolt visual acuities were below 20/20 in patients with SJS. To allow comparisons of timewise FVA changes between patients with SJS, patients with SS, and normal subjects, logarithm of minimal angle of resolution (logMAR) values of the FVA scores at 10, 20, and 30 seconds were divided by the logMAR baseline VA score. The lowest logMAR VA score was set at 2.7 for calculation of the FVA ratio: (2.7 − FVA at 10, 20, or 30 seconds)/(2.7 − baseline VA). The ratio allowed comparison of FVA at the testing points of 10, 20, and 30 seconds between the three groups.

A *t* test was performed for the comparison of FVA ratio scores at 10, 20, and 30 seconds of testing among patients with SJS and SS and normal controls subjects, as well as comparison of visual maintenance ratio (VMR). The relation between clinical ocular surface findings and FVA was investigated by multiple linear regression analysis. A probability level of $P < .05$ was considered statistically significant. SPSS software (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis.

RESULTS

THE MEAN SCHIRMER TEST VALUES WERE 10.3 ± 9.6 MM IN patients with SJS, 4.38 ± 3.66 mm in patients with SS, and 14.2 ± 9.4 mm in healthy control subjects. The Schirmer test values were higher in patients with SJS as compared with patients with SS; 56.9% of the patients with SJS had Schirmer test values >5 mm.

The average logMAR FVA showed a timewise decrease during testing in patients with SJS and SS and in normal subjects. Figure 2 shows the results of dynamic changes of VA. The timewise change of FVA was similar in patients with SJS and patients with SS, and the timewise decline of FVA was greater in patients with SS and patients with SJS compared with normal subjects. Mean logMAR FVA scores were lower in eyes of patients with SS and patients with SJS at 10, 20, and 30 seconds. The mean baseline VA score was lower in eyes of patients with SJS compared with control subjects.

Table 2 shows changes of VMR at 10, 20, and 30 seconds. VMR of patients with SJS were significantly lower compared with the normal subjects and patients with SS at 10, 20, and 30 seconds ($P < .05$).

Figure 3 shows the average severity scores of clinical findings in patients with SS and SJS. The mean clinical severity scores for trichiasis, symblepharon, meibomian gland dysfunction, corneal conjunctivalization, corneal opacity, and corneal vessels were markedly higher in patients with SJS compared with patients with SS. A total of 65.0% of the patients with SJS had grade 1 to 3 central corneal opacities. A total of 49.3% of the patients with SJS had grade 2 to 3 central vessels involving the central corneal area. A total of 58.8% of the patients with SJS had grade 0 to 2 corneal conjunctivalization not involving the pupillary area. A total of 42.6% of the patients with SJS had milder grade 0 to 1 meibomian gland dysfunction in this study.

Table 3 shows the relation of FVA at 10 seconds and the average scores of seven items of clinical findings. Dependent variable was logMAR FVA at 10 seconds, and independent variables were seven items of clinical findings. The results of multiple linear regression analysis

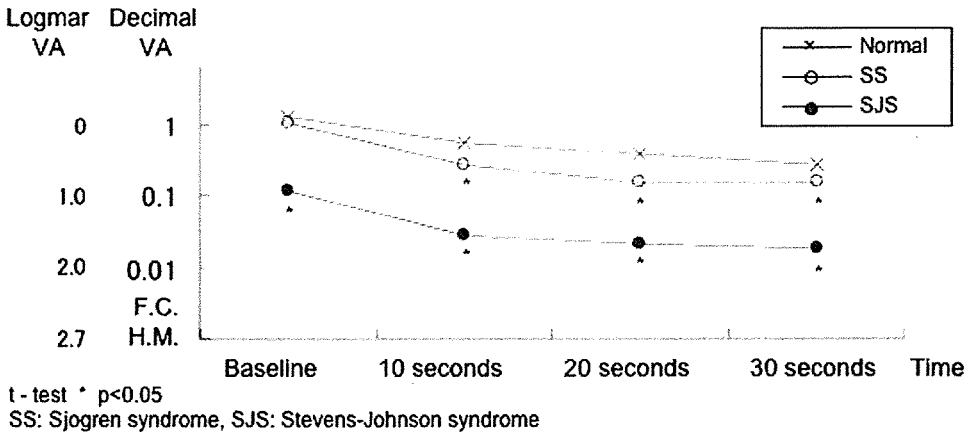

FIGURE 2. Dynamic changes of visual acuity (VA). Average logarithm of minimal angle of resolution (logMAR) functional visual acuity (FVA) showed timewise decrease during testing in patients with Stevens-Johnson syndrome (SJS), patients with Sjögren syndrome (SS), and normal subjects, but the time-wise decline of FVA was greater in patients with SS and SJS compared with normal subjects.

TABLE 2. Changes of Visual Maintenance Ratio

Patient	n	At 10 Seconds	At 20 Seconds	At 30 Seconds
Normal	20	0.87 ± 0.06	0.82 ± 0.08	0.77 ± 0.09
SS	40	0.79 ± 0.10*	0.71 ± 0.09*	0.70 ± 0.09
SJS	68	0.61 ± 0.26*	0.55 ± 0.09*	0.55 ± 0.18*

SS = Sjögren syndrome; SJS = Stevens-Johnson syndrome.
† By *t* test.
*$P < .05$.

between the clinical findings and mean logMAR FVA showed a relation between the presence of corneal opacity and vessels and the 10-second FVA scores.

DISCUSSION

ALTHOUGH STANDARD VA TESTING IS A GOOD MEASURE of one aspect of visual function, contrast sensitivity and glare testing provide other important and detailed information on visual function. Recently, FVA testing described as "FVA without blinking" has been reported to be an important method of defining "detailed visual function."[8] This method has been shown to be efficient in the detection of "masked impairment of visual function" in patients with dry eye who complain of decreased VA despite normal conventional VA. The definition of FVA testing has been suggested to be an important indication of an individual's performance in relation to certain daily activities involving visual performance. Vision-targeted health-related quality-of-life assessments quantify an aspect of dry eye disease distinct from "irritative dry eye symptoms or dry eye signs," which may not be measurable. The concept of FVA was first introduced by Goto and associates,[8] who previously reported abnormalities of FVA in subjects with dry eye. The major drawbacks in that study were the subjectivity of the method of measurement and uncertainty of the timing of FVA measurements because the VA measurements were carried out with conventional Landolt charts. Ishida and associates[13] also reported that FVA in dry eyes was markedly lower than in control subjects, and that FVA after punctum plug insertion greatly improved. FVA has also been reported to be effective in evaluating dynamic visual function changes after laser-assisted in situ keratomileusis.[20]

In this study, we aimed to study the changes of FVA in patients with SJS, comparing the findings with the results of patients with SS and with healthy control subjects. We chose patients with SJS because SJS is known to be associated with severe dry eyes and because of our interest in investigating the extent of differences of the tear functions and dynamic VA from patients with SS. We also assigned clinical severity scores to the examined ocular surface findings and studied the impact of clinical ocular surface findings on FVA. The FVA showed a similar timewise decline both in patients with SS and SJS. The dynamic VA decline was markedly different in both patient groups compared with healthy control subjects. It should be noted that the baseline conventional and FVA scores were similar in patients with SS and in control subjects. The baseline VA scores were much lower in patients with SJS. Statistical comparisons of VA scores in three groups with different baseline acuities and at designated testing times such as at 10, 20, or 30 seconds would have led to a biased interpretation of the functional VA results from patients with SJS as being statistically low.

In order to evaluate the decline of dynamic VA in subjects with different initial baseline VA, and to avoid the aforementioned biases, we devised VMR software, which measures the ratio of logMAR FVA at 10, 20, and 30 seconds to the baseline best-corrected logMAR con-

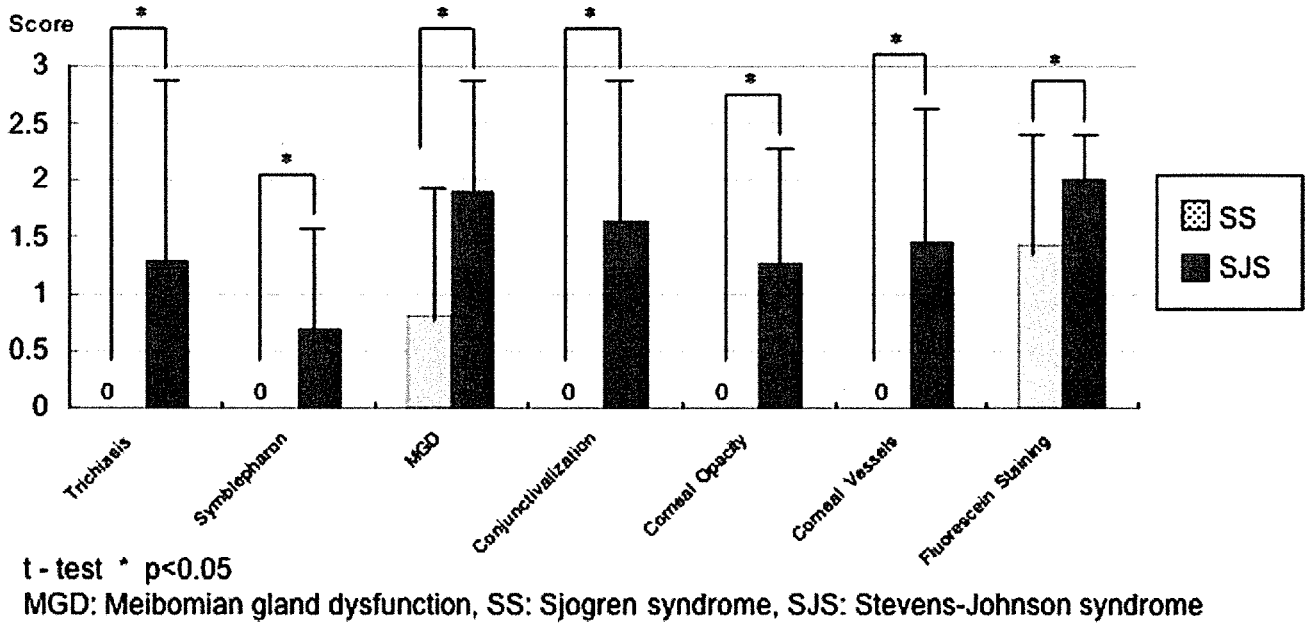

FIGURE 3. Mean clinical severity scores for trichiasis, symblepharon, Meibomian gland dysfunction (MGD), corneal conjunctivalization, corneal opacity, and corneal vessels, were much higher in patients with Stevens-Johnson syndrome (SJS) compared with patients with Sjögren syndrome (SS).

TABLE 3. Relation Between LogMAR Functional Visual Acuity and Clinical Finding Severity by Multiple Linear Regression Analysis in Patients With Stevens-Johnson Syndrome

Characteristic	Standard Partial Regression	*P*
Corneal opacity	0.50	.004*
Corneal vessel	0.29	.01*
Trichiasis	−0.13	.20
Meibomian gland dysfunction	0.08	.41
Conjunctivarization	0.06	.65
Symblepharon	−0.05	.58
Fluorescein staining	0.05	.58

LogMAR = logarithm of minimal angle of resolution.
t test $*p < 0.05$
† By *t* test. Dependent variables FVA at 10 seconds. Conditioned multiple correlation coefficient = 0.55.
$*P < .05$.

ventional Landolt VA. The mean VMRs at 10, 20, and 30 seconds were far lower in patients with SJS compared with patients with SS and normal control subjects, indicating poorer visual maintenance in patients with SJS despite a similar declining pattern of dynamic VA observed in patients with SS. We thus recommend VMR calculation for comparisons of groups with different baseline VA scores; the VMR calculations may be inserted as a database into the FVA measurement system software.

It should also be noted that 57% of patients with SJS in this study had relatively higher Schirmer test scores, and that patients with SJS did not all have very severe dry eyes. Despite better Schirmer test scores in approximately 60% of patients with SJS, the VMR was far lower in patients with SJS compared with patients with SS, which suggested the possibility that the FVA examination may reflect the ocular surface clinical findings. Therefore, we looked into the relation between the clinical severity scores of the examined ocular surface findings and FVA at 10 seconds. We chose the 10 second examination point for this comparison because it is our experience that 10 seconds of testing is sufficient to delineate the differences between normal and dry eyes, as we previously reported.[13] The mean clinical ocular surface finding severity scores in relation to all examined parameters were much higher in patients with SJS compared with patients with SS. We found a relation between the 10 second FVA and corneal opacity, as well as the presence of corneal vessels that we think were due to the involvement of the central corneal area by opacity or new vessels in most of the patients. Although not found to be correlated with FVA when examined alone, tear instability resulting from the combination of coexisting conjunctivalization, meibomian gland disease, trichiasis, and lid disorders with symblepharon in patients with SJS with normal tear quantity may explain the FVA decrease over 30 second testing. Other untested factors in this study, such as differences of ocular surface mucin expressions, tear film lipid, and protein and inflammatory cytokine profiles between patients with SJS and with SS, may explain the dissimilarities of the FVA patterns.

We did not find a marked relation between baseline FVA scores, conjunctivalization, and Meibomian gland dysfunction (MGD) as a result of the presence of milder

MGD in this series of patients and predominance of peripheral conjunctivalization not involving the central pupillary area. Interestingly, we did not find any relation between the FVA scores and fluorescein staining severity scores in patients with SJS this time. Our results suggest that corneal lesions involving the optical axis rather than punctuate staining seem to have more effect on the FVA scores. However, this assumption should be retested in a larger group of patients. Moreover, changes of FVA before and after treatment directed for central corneal opacities in various ocular surface diseases should be investigated in future trials. Prospective future studies in SS, non-SS, and severe dry eyes looking into the correlation of FVA scores with tear quality, quantity, ocular surface staining scores, and lipid layer interferometry grades are be essential and will provide very interesting information.

It should be noted that a topical anesthetic agent was used in this study to minimize discomfort and to prevent reflex tearing and blinking. The use of a topical anesthetic agent may introduce new variables of toxicity and tolerance of toxic medications by the ocular surface in patients with dry eye. We think that future tests should be performed for 10 seconds without an anesthetic agent. In addition, studies looking into the correlation between FVA scores and severity of dry eye–related visual symptoms as assessed by visual analog scale scores would also provide invaluable information. Worsening of functional VA seems to be a common complaint in subjects with dry eye, and we think the ability to test functional VA would be helpful in patient evaluation and follow-up of treatment effects. However, more work is needed to demonstrate the usefulness and validity of this new technology.

In summary, we found the FVA measurement system to be a useful tool in the assessment of dynamic VA changes in subjects with dry eye and in normal subjects, and a promising tool for evaluating the effect of clinical ocular surface findings and their severity on dynamic VA.[16]

THIS STUDY WAS SUPPORTED BY AN OFFICIAL GRANT FROM the Japan Ministry of Health and Welfare, Chiyodaku, Tokyo, Japan. The authors indicate no financial conflict of interest. Involved in design and study, management, analysis and interpretation of data, and preparation of the data (M.K., M.D., M.Y., C.S., S.K., J.S., K.T.); Involved in collection of data (M.K., M.D., M.Y., C.S., S.K., J.S., K.T.); and involved in the review and approval of the final manuscript (M.K., M.D., M.Y., C.S., S.K., J.S., K.T.).

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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

Accepted for publication Jul 27, 2006.

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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.

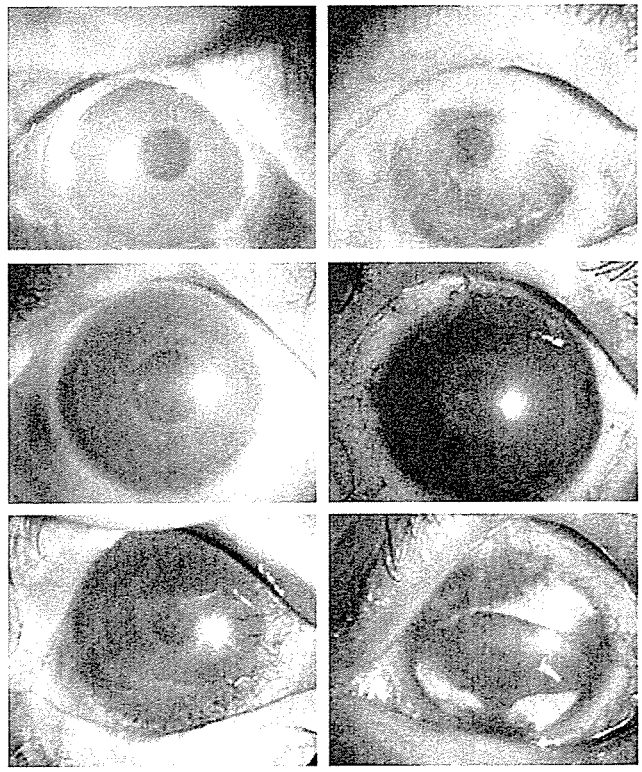

FIGURE 1. Preoperative and postoperative appearance of cultivated (left) and conventional limbal stem cell transplantation (right). (Top) Preoperative appearance. (Middle) Postoperative appearance at two months. (Bottom) Postoperative appearance at four years.

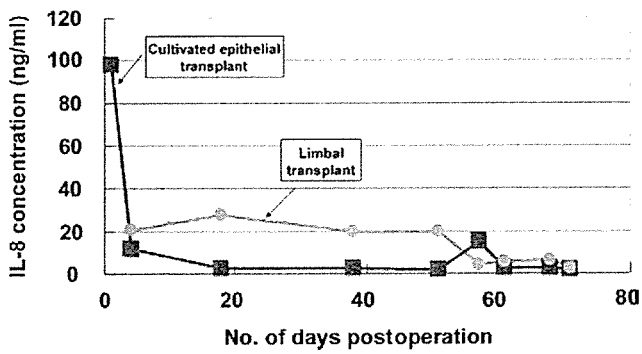

FIGURE 2. Cultivated and conventional limbal stem cell transplantation, pre- and postoperative interleukin (IL)-8 concentration in tears. A faster decrease in IL-8 levels was noted after cultivated epithelial transplantation compared with conventional limbal transplantation.

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.

THIS STUDY WAS SUPPORTED IN PART BY GRANTS-IN-AID for scientific research from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan; Research Grant from the Ministry of Health, Labour and Welfare, Tokyo, Japan; Intramural Research Fund from Kyoto Prefectural University of Medicine, Kawaramachi-dori, kamigyo-ku, Kyoto, Japan. The authors indicate no financial conflict of interest. Involved in design and conduct of study (L.P.K.A., C.S., N.K., T.S., T.I., S.K.); Involved in collection, management, analysis and interpretation of data, and preparation of the data and revision including references (L.P.K.A., C.S., N.K., T.S., T.I., S.K.); Involved in collection of data (L.P.K.A., C.S., N.K.); and involved in management, statistical analysis and interpretation of the data, and preparation of the manuscript (L.P.K.A., C.S., N.K., T.S., T.I., S.K.).

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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.)

Accepted for publication Aug 21, 2006.

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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.

BJO Online First, published on February 21, 2007 as 10.1136/bjo.2006.113449

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

(Running title : TLR3 polymorphisms in SJS)

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Keywords :
Stevens-Johnson syndrome (SJS), Toxic epidermal necrolysis (TEN),
Ocular surface complications, TLR3, Polymorphisms

Word count: 1913 words excluding title page, abstract, references, figures and tables

Abstract

Background: 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, we considered the possibility that there is an association between SJS/TEN and a disordered innate immune response. The first line of defense against infection is comprised of evolutionarily conserved sets of molecules, the Toll like receptors (TLRs). TLR3 recognizes double-stranded (ds) RNA associated with viral infections.

Methods: The Japanese Single Nucleotide Polymorphisms (JSNP) database reports 7 polymorphisms consisting of 7 SNPs in the human TLR3 gene; 3 of the 7 SNPs are coded in exon regions, i.e. 293248A/G, 293391A/G, and 299698T/G, the other 4 are coded in intron regions, i.e. 294440G/C, 294732C/T, 208036T/C, and 298054C/T. We analyzed these 7 SNPs in 57 Japanese SJS/TEN patients with ocular surface complications and 160 Japanese healthy controls.

Results: We found that SNP 299698T/G and the genotype pattern of 293248A/A and 299698T/T strongly associated with SJS/TEN.

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

Introduction

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 rate is 3 and 27%, respectively.[3] Although SJS and TEN are rare, they carry high morbidity and mortality rates and often result in severe handicaps such as visual loss. 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 for 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, SJS/TEN patients manifest vesiculobullous skin lesions, severe conjunctivitis, and persistent corneal epithelial defects due to 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, ankyloblepharon, and in some instances, keratinization of the ocular surface, persist (Fig.1A) despite the healing of the skin lesions.[4] We observed that more than 95% of SJS/TEN patients 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 etiologic factor in SJS/TEN.[5] In addition, it is noteworthy that SJS/TEN patients often had the prodromata, including nonspecific fever, coryza, and sore throat, that closely mimic upper respiratory tract infections commonly treated with antibiotics. These prodromata were evident from the clinical records of our SJS/TEN patients. Mycoplasma pneumoniae was responsible in 5 of 17 cases of childhood SJS[6] and a viral etiology 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 MRSA or MRSE,[9] we considered the possibility that there is an association between SJS/TEN and a disordered innate immune response. We postulated that viral infection and/or drugs may 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 defense against infection is comprised of evolutionarily conserved sets of molecules, the Toll like receptors (TLRs). The triggering of TLRs results in the secretion of anti-bacterial peptides and pro-inflammatory cytokines. The inflammatory response results in the recruitment of cells of adaptive immunity to initiate clearance of the pathogens. TLR3 recognizes double-stranded (ds) RNA associated with viral infections, a component of the life-cycle of most viruses.[10] As functional deterioration of TLR3 may predispose individuals to increased susceptibility to viral infections, the detection of TLR3 polymorphisms may yield critical information for risk assessment regarding susceptibility to microbial infections in the context of SJS/TEN.

To date, there have been no reports on the genetic loci of TLR3 in subjects with SJS/TEN. Therefore, we performed SNP association analysis of the TLR3 gene, which maps to chromosome 4q35. The Japanese Single Nucleotide Polymorphisms (JSNP) database reports 7 polymorphisms consisting of 7 SNPs in the human TLR3 gene; 3 of the 7 SNPs are coded in exon regions, i.e. 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), the other 4 are coded in intron regions, i.e. 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 analyzed these 7 SNPs in 57 Japanese SJS/TEN patients with ocular surface complications and 160 Japanese healthy controls. We found that SNP 299698T/G and the genotype pattern of 293248A/A and 299698T/T strongly associated with SJS/TEN.

Materials and Methods

Patients

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

For SNPs analysis, we enrolled 57 patients with SJS/TEN in the chronic or sub-acute 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 2 mucosal sites including the ocular surface. The controls were 160 healthy volunteers. All participants and volunteers were Japanese residing in Japan. The average age of the patients involved in this study was 45.2 ± 17.5 (SD) years, and that of the controls was 36.2 ± 11.5 (SD) years. The numbers of male/females in the patient and control groups were 24 / 33 and 57/ 103, respectively.

SNPs 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, 5'- TTACCTTCTGCTTGACAAAGGG -3' (sense) and 5'-TGCATTTGAAAGCCATCTGC -3' (antisense) for rs. 3775296. All primers but for rs. 3775290 and rs. 3775291, were those recommended in the JSNP database. Genomic DNA was isolated from human peripheral blood at SRL Inc. (Tokyo, Japan). PCR amplification was with DNA polymerase (Takara; Shiga, Japan) for 35 cycles at 94°C for 1 min, annealing at 60°C for 1 min, and 72°C for 1 min on a commercial PCR machine (GeneAmp; Perkin-Elmer Applied Biosystems). The PCR products were reacted with BigDye Terminator v3.1 (Applied Biosystems) and sequence reactions were resolved on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Statistical methods

Alleles were counted manually. Genotype patterns were also 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% confidence intervals (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

A summary of our case-control association study on the 7 SNPs genotyped to TLR3 is shown in Table 1.

Table 1

Genotype frequencies of TLR3 SNPs among Japanese SJS/TEN patients and healthy controls

	Control (%) (N=160)	SJS/TEN (%) (N=57)	Allele 1 v.s. Allele 2 P-value(χ^2) OR (95%CI)	Genotype 11 vs 12+22 P-value(χ^2) OR (95%CI)	Genotype 11+12 vs 22 P-value(χ^2) OR (95%CI)
293248 (rs.3775290)					
11 GG	63 (39.4)	19 (33.3) 25	0.110	0.419	**0.046**
12 GA	78 (48.8)	(43.9)	-	-	**0.456**
22 AA	19 (11.9)	13 (22.8)	(-)	(-)	**(0.209-0.997)**
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)**

All but SNP 294732C/T were in Hardy-Weinberg equilibrium in both our SJS/TEN patients 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 p-value = 0.007, OR = 0.22). However, when we corrected the p-value for the number of alleles tested (n=7), the results ceased to be 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 analyzed the genotype pattern of SNPs 299698T/G and 293248A/G and found that it (293248A/A - 299698T/T) also strongly associated with SJS/TEN in Japanese patients (χ^2 test, p = 0.0006, OR = 5.5, 95% CI = 1.9 -15.8) (Table 2). This association was stronger than observed for the single locus (299698T/G).

Table 2

Pattern structures and frequency of SNPs293248A/G and 299698T/G

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

Our results suggest that polymorphisms in the TLR3 gene may be associated with SJS/TEN in the Japanese population. We hypothesized that viral infection and/or drugs may 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 may play a role in an integrated etiology 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 (RV) 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 RV 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.[12,13,14] SJS/TEN may be the consequence of exposure of genetically susceptible individuals to specific environmental precipitants. A report from the United States showed that the HLA-B12 (HLA-Bw44) antigen was significantly increased in Caucasian patients with SJS with ocular involvement.[15] Analyses of TEN

patients 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.[19,20,21,22,23] Heretofore, it was not recognized that in the pathophysiology of SJS/TEN, innate immunity plays a critical role in the bridging between the acute response to invading non-self molecules and chronic local immune inflammation.

We previously reported that while human corneal epithelium harbors 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 mRNA level (data not shown). We reported that cell-surface TLR3 of human corneal epithelial cells responds to virus dsRNA-mimic polyI:C to generate pro-inflammatory cytokines and IFN-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 SJS/TEN patients and TLR3 gene polymorphisms. This raises the possibility that abnormalities of TLR3 on the ocular surface may 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 may be associated with type 1 diabetes in South African blacks.[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 may constitute a link between the environment and the adaptive immune system.

Acknowledgements

We thank Ms. C. Mochida for her technical assistance.

Supported in part by grants-in-aid for scientific research from the Japanese Ministry of Health, Labour and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology, CREST from JST, a research grant from the Kyoto Foundation for the Promotion of Medical Science and, the Intramural Research Fund of Kyoto Prefectural University of Medicine.

Figure legends

Figure 1

SJS/TEN with ocular complications

A. Ocular surface complications such as conjunctival invasion into the cornea, symblepharon, ankyloblepharon, and sometimes keratinization of the ocular surface, persist in some SJS/TEN patients in the chronic stage. Conjunctival invasion results in severe vision loss.

B. Transformed fingernails of SJS/TEN patients with ocular complications. Many SJS/TEN patients 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 male 6 years after onset (chronic stage).

Figure 2

Genomic organization of the TLR3 gene on chromosome 4q35

The genomic organization of the gene was derived from the Japanese Single Nucleotide Polymorphism (JSNP) database. Seven polymorphisms consisting of 7 SNPs have been reported in the human TLR3 gene in the JSNP database; 3 of 7 SNPs are coded in exon regions, i.e. 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), the other 4 are coded in intron regions, i.e. 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).

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