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

(Running title : TLR3 polymorphisms in SJS)

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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.

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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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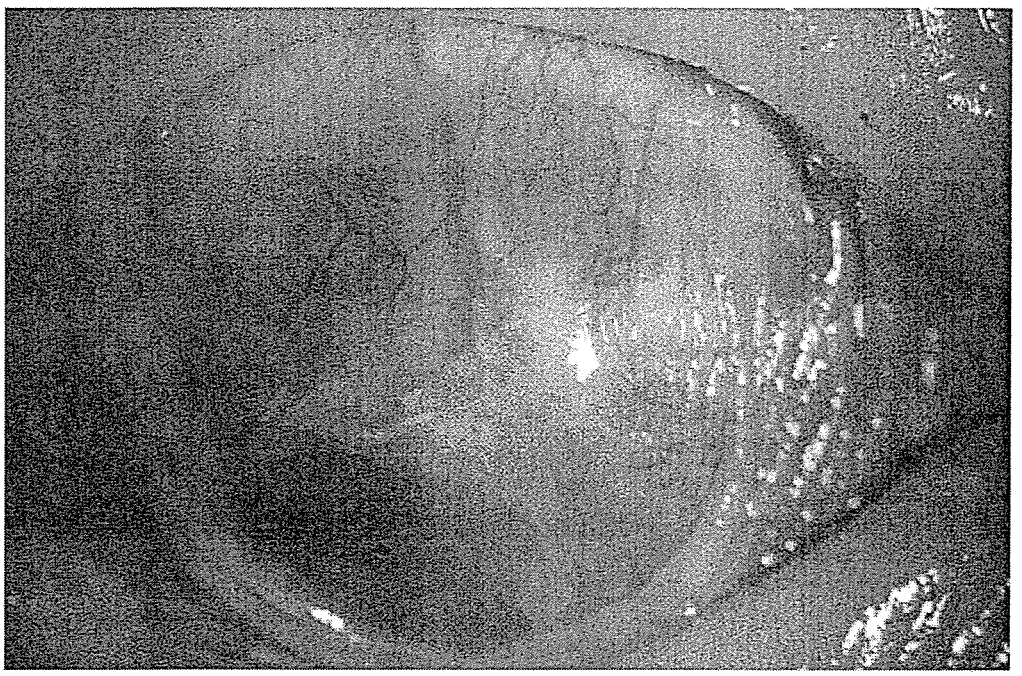
Figure1

A.



B.

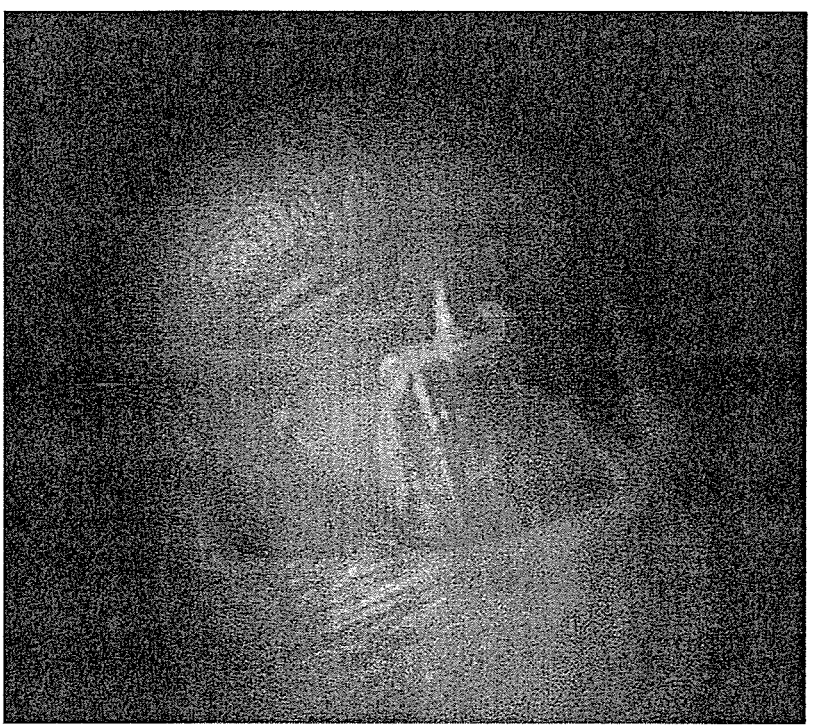
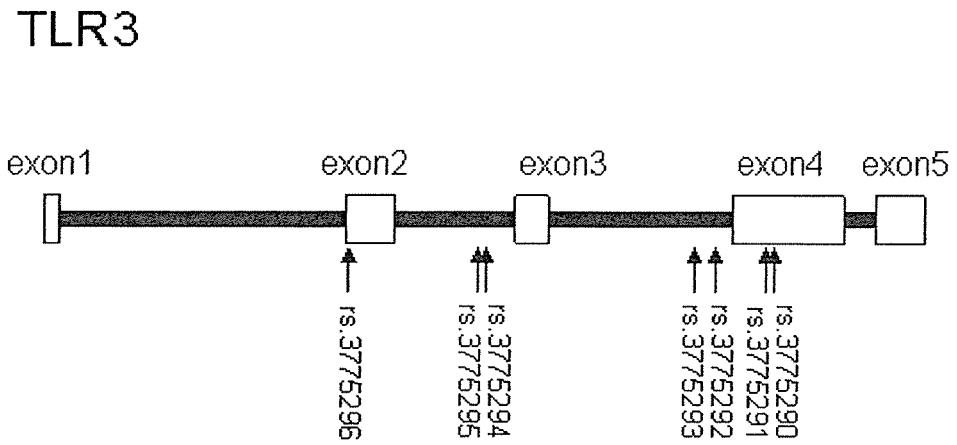


Figure2


TLR3
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A NEW GRADING SYSTEM FOR THE EVALUATION OF CHRONIC OCULAR MANIFESTATIONS IN PATIENTS WITH STEVENS-JOHNSON SYNDROME

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Running head: A new way to grade ocular manifestations in Stevens-Johnson syndrome

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ABSTRACT

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 complications (trichiasis, mucocutaneous junction involvement, meibomian gland involvement, punctal damage).
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 significantly correlated with 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 ($R= 0.806$, $p<0.0001$). Multivariable regression analysis showed that corneal neovascularization, opacification, keratinization, and cataracts significantly affected logMAR ($p<0.0001$, $p<0.0001$, $p=0.0142$, $p=0.0375$, respectively)
Conclusions: We 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 was significantly correlated 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.

INTRODUCTION

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 resolution 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 is often 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, as no clear diagnostic criteria have been established, reaching a definitive diagnosis can be difficult. Roujeau et al.,[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 two separate clinical entities that differed with respect to histopathologic changes and etiology. A large international case-control study, called the Severe Cutaneous Adverse Reaction (SCAR) study, prospectively evaluated the validity of this clinical distinction; its results strongly supported 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 pathology of skin lesions present in the "acute stage". However, patients often present to ophthalmologists in the late stage of the disease with chronic cicatricial complications, after resolution of the dermatological changes, and it can be difficult to elicit the original clinical manifestations used to distinguish between EMM and SJS/TEN from patients seen many years after disease-onset. Therefore, from the ophthalmologist's perspective, ocular surface diseases arising from EM, SJS, or TEN are often collectively regarded 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 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 aim of this study was 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. As 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 Tokyo Medical Center. All patients with chronic ocular complications from SJS who were referred to these centers between April 2003 to March 2005 were prospectively evaluated 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 institute; 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 was also taken by the attending physician. A drug was considered a possible etiologic agent if it had been taken shortly before the onset of symptoms, i.e. 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 - 3 depending on the severity of involvement. The complications were broadly classified as corneal complications comprised of superficial punctate keratopathy, epithelial defect, loss of the palisades of Vogt, 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 system were used to evaluate the nature of the ocular complications in these patients:

Corneal complications

1. Severity of superficial punctate keratopathy (SPK). 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. While Miyata *et al.*[12] 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 - 3: A1D1 was scored as 0; A1D2 or A2D1 as 1; A1D3, A2D2, or A3D1 as 2, and A2D3, A3D2, or A3D3 as 3.

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

3. *Loss of the palisades of Vogt.* The extent of the loss of the limbal palisades of Vogt (POV) was graded from 0 - 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.

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

5. *Corneal neovascularization.* The extent of corneal neovascularization was scored from 0 - 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.

6. *Corneal opacification.* The severity of corneal opacification was graded from 0 - 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).

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

Conjunctival complications

8. *Conjunctival hyperemia.* Conjunctival hyperemia was graded from 0 - 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).

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

Eyelid complications

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