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Review Article

Genetic susceptibility for Stevens-Johnson syndrome/Toxic epidermal necrolysis with mucosal involvements

Mayumi Ueta[1, 2, *)]

[1)]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[2)]Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute inflammatory vesiculobullous reactions of the skin and mucous membranes including oral cavity and ocular surface.

Single nucleotide polymorphism (SNP) association analysis of SJS/TEN patients with severe mucosal involvements (MI) revealed that TLR3 SNPs and IL4R SNP Gln551Arg were significantly associated with SJS/TEN with MI. We also found that *PTGER3* SNPs were associated with the SJS/TEN using genome-wide association study (GWAS). The expression of EP3 (protein of *PTGER3 gene*) was greatly reduced in the conjunctival epithelium of the SJS/TEN patients compared to the controls. About 80% of our SJS/TEN patients with MI had used cold medications before the onset of their disease, and cold medicines including non-steroid anti-inflammatory drugs (NSAIDs) could inhibit the production of the EP3 ligand PGE_2. Thus, EP3 may contribute to the development of SJS/TEN with MI.

We also found that a combination of *TLR3* and *PTGER3* SNPs could raise the genetic susceptibility of SJS/TEN with MI, and that there were functional interactions between TLR3 and EP3, the protein of *PTGER3*. This suggests that a lack of balance between TLR3 and EP3 may trigger mucosal inflammation at sites such as the ocular surface.

Elsewhere we documented that in Japanese patients HLA-A*0206 is strongly associated with the SJS/TEN. We also reported multiplicative interaction(s) between HLA-A*0206 and TLR3 SNPs in patients with the SJS/TEN.

Together, our observations suggest that not only environmental- but also genetic factors, including epistatic interactions, play a role in an integrated etiology of SJS/TEN with MI.

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* Correspondence should be addressed to:
Mayumi Ueta, Department of Ophthalmology, Kyoto Prefectural University of Medicine, Hirokoji, Kawaramachi, Kamigyo-ku, Kyoto 602-0841, Japan. Phone: +81-75-251-5578, Fax: +81-75-251-5663, E-mail: mueta@koto.kpu-m.ac.jp

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Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN)

Stevens-Johnson syndrome (SJS), an acute inflammatory vesiculobullous reaction of the skin and mucous membranes, was first described in 1922 by the American pediatricians Stevens and Johnson[1]. They encountered 2 boys aged 7 and 8 who presented with an extraordinary, generalized eruption with persistent fever, inflamed buccal mucosa, and severe purulent conjunctivitis that progressed to severe visual disturbance[1]. Other pediatricians reported that SJS was associated with infectious agents such as *Mycoplasma pneumoniae*[2] or a viral etiology[3].

According to dermatologists, SJS and its severe variant toxic epidermal necrolysis (TEN) are life-threatening severe adverse drug reactions characterized by high fever, rapidly developing blistering exanthema of macules accompanied by skin detachment and mucosal involvement[4].

The annual incidence of SJS and TEN has been estimated as 1-6 and 0.4-1 cases per million persons, respectively[5, 6]. Although very rare, the mortality rate of SJS and TEN is 3% and 27%, respectively[7] and they often result in vision loss. While the pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established, the extreme rarity of cutaneous and mucosal reactions due to drug therapies led us to suspect individual susceptibility.

According to a European-American consensus classification, SJS is recorded in patients with manifest detachment of less than 10% of the body surface area (BSA) and widespread erythematous or purpuric macules or flat atypical targets. Overlapping SJS/TEN is diagnosed when detachment involves 10-30% of BSA and there are widespread purpuric macules or flat atypical targets. TEN is the diagnosis in patients with spots who show more than 30% detachment with widespread purpuric macules or flat atypical targets. TEN without spots is recorded in patients with more than 10% detachment, large epidermal sheets, and no purpuric macules[5].

In Japan, a different classification was proposed. SJS is diagnosed in patients with less than 10% BSA detachment, widespread blistering exanthema of macules, and atypical target-like lesions accompanied by MI. A diagnosis of TEN is made when BSA detachment exceeds the less than 10% seen in SJS[8]. For a diagnosis of SJS there must be MI and cutaneous lesions; MI may or may not be present in patients with a diagnosis of TEN.

Dermatologists tend to see patients with SJS/TEN in the acute stage. On the other hand, ophthalmologists encounter SJS/TEN not only in the acute- but more often in the chronic stage. When a differential diagnosis of SJS or TEN is complicated because the vesiculobullous skin lesions present in the acute stage have healed. Consequently, ophthalmologists have tended to report both SJS and TEN broadly as SJS. The ophthalmological diagnosis of SJS/TEN (SJS in the broad sense) was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites including the ocular surface[9-14].

In the acute stage, SJS/TEN patients with MI manifest severe conjunctivitis, corneal/conjunctival epithelial defects, and alopecia with vesiculobullous lesions of the lip, oral cavity, and skin (Fig. 1A, B). In the chronic stage, ocular surface complications such as severe dry eye, symblepharon, and trichiasis persist despite the healing of the skin lesions (Fig. 1C). In some patients with corneal epithelial stem cell deficiency (Fig. 1C) conjunctival invasion into the cornea may have progressed to keratinization of the ocular surface and

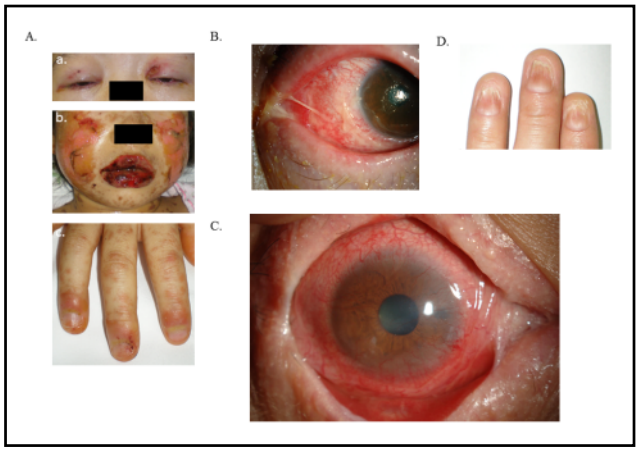

Fig. 1 Stevens-Johnson syndrome (SJS) with severe mucosal involvement

(A)Typical features of SJS/ TEN in the acute stage.
a. Ocular surface inflammation with conjunctivitis and eyelid swelling.
b. The face manifests swollen and crusted lips, blisters, and erosion of the skin.
c. Paronychia
(Reprinted with permission from Ueta et al.[18])
(B)Ocular surface inflammation of SJS results in severe conjunctivitis, pseudomembrane, and epithelial defect.
(C)Ocular surface complications in the chronic stage are conjunctival invasion into the cornea, symblepharon, trichiasis, and dry eye.
(D)Transformed fingernails in the chronic stage.
(Reprinted with permission from Ueta et al.[19])

these patients present with severe visual disturbance. SJS/TEN with MI is one of the most devastating ocular surface diseases leading to corneal damage and loss of vision. We also observed that more than 95% of patients with SJS/TEN with MI had lost their fingernails in the acute- or subacute stage and that many of them continue to have transformed nails even after healing of the skin lesions (Fig. 1D)[10, 13].

SJS/TEN with MI and the role of disordered innate immunity

Drugs, particularly antibiotics and cold medicines including NSAIDs and multi-ingredient cold medications, are an accepted etiologic factor in SJS/TEN[4, 8]. Many SJS/TEN patients with MI experienced prodromata including nonspecific fever, coryza, and sore throat that closely mimic upper respiratory tract infections commonly treated with antibiotics and cold medicines[6, 13, 15]. In fact, 131 of our 162 patients (81%) developed SJS/TEN with MI after drug treatments for the common cold with antibiotics, cold remedies, and/or NSAIDs[13, 15].

Moreover, the detection rate for methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (MRSA, MRSE) was higher on the ocular surface of SJS/TEN patients with MI than in patients with other devastating ocular surface disorders[16]. Given the association between the onset of SJS/TEN with MI and infection, and the opportunistic infection of ocular surfaces by bacteria such as MRSA and MRSE, we posited an association between SJS/TEN with MI and a disordered innate immune response[10, 13, 17-19]. We postulated that microbial infection with, for example, mycoplasma or viruses, and/or certain drugs trigger a disorder in the host's innate immune response and that this event is followed by aggravated inflammation of the mucous membranes including the ocular surface and the skin. We further hypothesized that an abnormality in the innate immunity of the ocular surface results in ocular surface inflammation[18, 19] because inflammatory bowel disease is thought to result from an abnormal response to gut microbiota[20].

Elsewhere we reported that IκB$\zeta^{-/-}$ mice expressly exhibit severe, spontaneous ocular surface inflammation accompanied by the eventual loss of almost all goblet cells[21] and that they also manifest perioral- and skin inflammation (Fig. 2A)[22] as do patients with SJS/TEN with MI[23, 24]. Moreover, as some IκBζ knock-out (KO) mice present with ocular surface inflammation with corneal opacity (Fig. 2A)[18] we considered them a suitable model for SJS/TEN with MI[18, 19, 21, 22].

IκBζ-KO mice also exhibit oral mucositis, and airway inflammation (Fig. 2B)[18]. Moreover, IκBζ/Stat6 double knockout (DKO) mice manifested severe dermatitis of the facial- and the abdominal skin; they also exhibited paronychia (Fig. 2C) as is seen in human SJS/TEN with MI[18].

IκBζ is induced by diverse pathogen-associated molecular patterns. It regulates NF-κB activity[25] and is important for Toll-like receptor signaling, which is essential for the innate immune response. The spontaneous inflammation of the ocular surface and oral cavity seen in IκBζ KO mice suggests that dysfunction/abnormality of innate immunity can lead to mucosal inflammation and we postulated that it may also play a role in human ocular surface inflammatory disorders.

While the presence of commensal bacteria such as *S. epidermidis* and *P. acnes* on the normal ocular surface does not elicit a response and no inflammation is induced[18, 19], a hyper-inflammatory reaction against bacteria may result in ocular surface inflammation. SJS/TEN patients with MI often develop severe ocular surface inflammation in the presence of bacteria such as MRSA or MRSE on their ocular surface. Elderly hospitalized patients do not exhibit severe ocular surface inflammation despite the presence of MRSA or MRSE on the ocular surface and the severity of ocular surface inflammation in SJS/TEN patients with MI is greatly reduced by treatment with antibiotics against MRSA or MRSE[18, 19].

Under the hypothesis of a relationship between a disordered innate immune response and SJS/TEN with MI we performed gene expression analysis of monocytes which play an essential role in innate immunity. When we incubated monocytes for 1 hr with or without lipopolysaccharide (LPS) we found that IL-4R gene expression was different in SJS/TEN patients with MI and the controls. Upon LPS stimulation it was down-regulated in patients and slightly up-regulated in the controls despite large individual differences with respect to LPS reactivity[17]. Our findings suggest that IL-4R expression is linked to innate immune responses and that the differences in IL-4R gene expression might play an important role in the pathophysiology of SJS/TEN with MI.

SNP association analysis by the candidate gene approach

SJS/TEN with MI can be induced by drugs. However, not all patients treated with drugs develop SJS/TEN with MI and but rare patients develop SJS/TEN with MI. To test our hy-

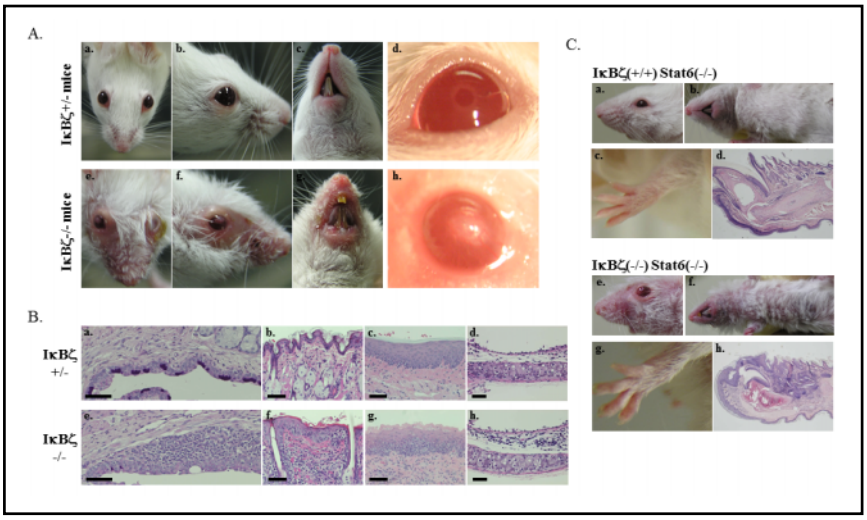

Fig. 2 Phenotype and histological findings in -KO- and IκBζ/Stat6-DKO mice, a suitable model for human SJS/TEN with severe mucosal involvement

(A)Phenotype of IκBζ KO mice

Photographs of the face and perioral skin of 32-week-old IκBζ$^{+/-}$ and IκBζ$^{-/-}$ mice taken 27 weeks after symptom onset. While IκBζ$^{+/-}$ mice were free of inflammation (a-d), IκBζ$^{-/-}$ mice exhibited a severe inflammatory phenotype. Their inflammation involved the ocular surface, eyelids, and perioral skin. Some of these mice manifested corneal opacity with ocular surface inflammation (e-h).

(B)Histological findings on the palpebral conjunctiva, perioral skin, oral mucosa, and trachea of IκBζ$^{+/-}$ and IκBζ$^{-/-}$ mice. We observed no pathological changes such as inflammatory phenotypes in IκBζ$^{+/-}$ mice (a-d).

However, the palpebral conjunctiva of an IκBζ$^{-/-}$ mouse (at 2 weeks after the onset of inflammatory symptoms) revealed heavy infiltration by inflammatory cells into the submucosa and loss of goblet cells (arrows in a) in the conjunctival epithelia (e). The perioral skin of the IκBζ$^{-/-}$ mouse showed hyperplasia and spongiosis in the epidermis including the hair follicles, inter- and intracellular edema in the epidermis, and heavy infiltration of the dermis by inflammatory cells (f). The oral mucosa of another IκBζ$^{-/-}$ mouse (at 9 weeks post-onset) revealed spongiosis in the epithelium and infiltration by inflammatory cells into the submucosa under the oral mucosal epithelia (g). In the trachea of another IκBζ$^{-/-}$ mouse (at 8 weeks post-onset) we found infiltration of inflammatory cells into the submucosa under the tracheal epithelia (h).

Each bar represents 50 μm

(C)Phenotype and histological findings in an IκBζ/Stat6 double-KO mouse.

No obvious dermatitis or paronychia was observed in Stat6 single-KO mice (a-d). However, in the IκBζ/Stat6-DKO mouse severe inflammatory symptoms were elicited on the ocular surface and not only the facial- but also the abdominal skin was involved (e, f). The IκBζ/Stat6 WKO mouse also manifested paronychia (g, h).

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pothesis that there is a genetic predisposition in individuals who develop SJS/TEN with MI we performed SNP association analysis using the candidate gene approach.

1)TLR3

We first analyzed SNPs of TLR3, which ligand is a viral double strand RNA (dsRNA), because there is an association between the onset of SJS/TEN with MI and infection. Many SJS/TEN patients with MI, experienced prodromata including nonspecific fever, coryza, and sore throat that closely mimic upper respiratory tract infections commonly treated with antibiotics[13]. Elsewhere we documented that the human ocular surface epithelium (corneal and conjunctival epithelium) harbors messages for most TLRs, that TLR3 is the most highly expressed TLR, and that the cell-surface TLR3 of human ocular surface epithelial cells responds to viral dsRNA-mimic polyI:C to generate pro-inflammatory cytokines and IFN-β (Ueta, 2005 #38;Ueta, 2010 #101) .

Analysis of the 17 SNPs of *TLR3* showed that 7 SNPs were associated with SJS/TEN with MI in 110 samples from patients and 206 control samples[26, 27]. There were associations with TLR3 rs.5743312T/T SNP (T/T vs T/C+C/C: p=2.5x10^{-6}, OR=7.4), TLR3 rs.3775296T/T SNP (T/T vs T/G+G/G: p=8.2x10^{-6}, OR=5.8), TLR3 rs.6822014G/G SNP (G/G vs G/A+A/A: p=1.2x10^{-4}, OR=4.8), TLR3 rs.3775290A/A SNP (A/A vs A/G+G/G: p=7.1x10^{-4}, OR=2.9), TLR3 rs.7668666A/A SNP (A/A vs A/G+G/G: p=1.2 x10^{-3}, OR=2.7), TLR3 rs.4861699G/G SNP (G/G vs G/A+A/A: p=4.2x10^{-4}, OR=2.3), and TLR3 rs.11732384G/G SNP (G/G vs G/A+A/A: p=8.5 x10^{-3}, OR=1.9) (Table 1)[27].

We hypothesized that microbial infection elicited by viruses and/or drugs may trigger a disorder in the host innate immune response and that this event is followed by aggravated mucosal inflammation of the ocular surface and skin. Our findings suggest that genetic and environmental factors play a role in an integrated etiology of SJS/TEN with MI and that there is an association between SJS/TEN with MI and disordered innate immunity[13, 18, 19, 26, 27].

Table 1 Association between TLR3 SNPs and SJS/TEN with mucosal involvement

rs number of SNP	Genotypes		Cases (n=110)	Controls (n=206) (%)	Allele 1 vs. Allele 2	Genotype 11 vs. 12+22	Genotype 11+12 vs. 22
					P-value[a] OR[b] (95%CI[c])	P-value[a] OR[b] (95%CI[c])	P-value[a] OR[b] (95%CI[c])
rs4861699	11	G/G	65/110 (59.1%)	79/206 (38.3%)	0.0016	$4.2x10^{-4}$	0.28
	12	G/A	36/110 (32.7%)	102/206 (49.5%)	1.80	2.32	1.55
	22	A/A	9/110 (8.2%)	25/206 (12.1%)	(1.25-2.59)	(1.45-3.72)	(0.70-3.45)
rs6822014	11	A/A	55/110 (50.0%)	127/206 (61.7%)	$8.9x10^{-4}$	0.046	$1.2x10^{-4}$
	12	A/G	37/110 (33.6%)	71/206 (34.5%)	0.54	0.62	0.21
	22	G/G	18/110 (16.4%)	8/206 (3.9%)	(0.37-0.78)	(0.39-0.99)	(0.09-0.49)
rs11732384	11	G/G	72/110 (65.5%)	103/206 (50.0%)	0.029	0.0085	0.88
	12	G/A	31/110 (28.2%)	89/206 (43.2%)	1.54	1.89	1.07
	22	A/A	7/110 (6.4%)	14/206 (6.8%)	(1.04-2.28)	(1.17-3.06)	(0.42-2.74)
rs3775296	11	G/G	49/110 (44.5%)	109/206 (52.9%)	0.0020	0.16	$8.2x10^{-6}$
	12	G/T	40/110 (36.4%)	89/206 (43.2%)	0.58	0.71	0.17
	22	T/T	21/110 (19.1%)	8/206 (3.9%)	(0.40-0.82)	(0.45-1.14)	(0.07-0.40)
rs5743312	11	C/C	52/110 (47.3%)	115/206 (55.8%)	0.0014	0.15	$2.5x10^{-6}$
	12	C/T	38/110 (34.5%)	85/206 (41.3%)	0.56	0.71	0.14
	22	T/T	20/110 (18.2%)	6/206 (2.9%)	(0.39-0.80)	(0.45-1.13)	(0.05-0.35)
rs7668666	11	C/C	36/110 (32.7%)	83/206 (40.3%)	0.0085	0.19	0.0012
	12	C/A	47/110 (42.7%)	101/206 (49.0%)	0.64	0.72	0.37
	22	A/A	27/110 (24.5%)	22/206 (10.7%)	(0.46-0.89)	(0.44-1.17)	(0.20-0.68)
rs3775290	11	G/G	38/110 (34.5%)	82/206 (39.8%)	0.016	0.36	$7.1x10^{-4}$
	12	G/A	45/110 (40.9%)	103/206 (50.0%)	0.66	0.80	0.35
	22	A/A	27/110 (24.5%)	21/206 (10.2%)	(0.48-0.93)	(0.50-1.29)	(0.18-0.65)

[a]*P*-value for allele- or genotype frequency comparisons between patients and controls using the chi-square test.
[b]OR, odds ratio.
[c]CI, confidence interval.
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2)IL-4R

We also examined SNPs of IL-4R genes because our gene expression analysis had shown that IL-4R gene expression was different in SJS/TEN patients with MI and the controls[17].

We compared Ile50Val (rs.1805010), Gln551Arg (rs.1801275), and Ser478Pro (rs.1805015) polymorphisms in Japanese patients with SJS/TEN with MI and healthy Japanese volunteers. Among the 3 SNPs of IL-4R, Gln551Arg showed a significant association[10, 12] with the allele frequency (A vs G, p value = 0.0046, OR = 2.3) and the dominant model (A/A vs A/G + G/G, p value = 0.0042, OR = 2.4) in the 110 patients and the 220 controls. With respect to Gln551Arg polymorphisms, Gln551- but not Arg551 alleles were significantly increased in SJS/TEN patients with MI[10, 12]. Others had shown that Arg551 alleles were significantly increased in patients with asthma[28] and atopy[29]. SJS/TEN with MI was associated with Gln551Arg, shown to have no effect on IgE synthesis[30], but not with Ile50Val and Ser478Pro associated with IgE synthesis[30, 31]. To investigate the relationship between serum IgE and SJS/TEN with MI we assayed total IgE. We found no significant difference between SJS/TEN patients with MI and the controls with respect to the incidence of high total serum IgE[10]. This indicates that serum IgE was not associated with SJS/TEN with MI and coincides with our earlier finding of an association between SJS/TEN with MI and Gln551Arg, which has no effect on IgE synthesis[10].

A strong genetic predisposition underlies the manifestation of allergic diseases such as asthma and atopy and IL-4R is a representative candidate gene[28-30]. While our results suggest an association with IL-4R gene polymorphism, Gln551Arg, in Japanese patients with SJS/TEN with MI, we posit that SJS/TEN with MI is different from allergic diseases because the ratio of each allele in the polymorphisms was the opposite of the ratio reported in atopy and asthma[10, 12].

As we also found that IL-4R-specific mRNA was downregulated in human ocular surface epithelial cells upon their stimulation with PolyI:C that mimics viral components, we suggest that IL-4R is linked with innate immunity.

Genome-wide association study (GWAS)

For a detailed understanding of the pathophysiology of SJS/TEN with MI we performed GWAS of more than 10^5 SNPs. GWAS permits the identification of genetic loci and genes associated with complex human traits without bias or *a priori* knowledge of the function or involvement of genes in the disease pathway. GWAS using the Affymetrix GeneChip mapping 500K array set detected 3 SNPs (rs1325975: chr6, rs17131450: chr1, rs11238074: chr11) that were significantly associated with SJS/TEN with MI[15]. Because SNPs rs1325975 and rs11238074 were from the "gene desert" region we focused on SNP rs17131450 which mapped close to the *PTGER3 gene* located in the 1p31 region of the human genome[15]. Based on our GWAS results we performed fine-mapping analysis of the *PTGER3* region using a custom DNA array to analyze the SNPs in and near *PTGER3*. We identified 5 other significantly associated ($p < 0.01$) SNPs, i.e. rs5702, rs1325949, rs7543182, rs7555874, and rs4147114[15]. One of the 6 SNPs in *PTGER3* (rs5702) was

Table 2 Association between EP3 SNPs and SJS/TEN with ocular complications

rs number of SNP	Frequency of genotypes (%)				Allele 1 vs. Allele 2	Genotype 11 vs. 12+22	Genotype 11+12 vs. 22
	genotypes		control	case	*P*-value[a] OR[b] (95%CI[c])	*P*-value[a] OR[b] (95%CI[c])	*P*-value[a] OR[b] (95%CI[c])
rs7555865	11	C/C	47.9	45.7	0.10	0.69	0.0083
	12	C/T	42.5	34.5	-	-	0.43
	22	T/T	9.6	19.8	-	-	(0.2-0.8)
rs17131450	11	C/C	87.8	76.7	0.00069	0.0086	0.0039
	12	C/T	11.8	18.1	0.41	0.46	0.08
	22	T/T	0.5	5.2	(0.2-0.7)	(0.3-0.8)	(0.01-0.7)
rs5702	11	C/C	49.3	64.7	0.059	0.0072	0.6
	12	C/T	43.0	25.9	-	1.88	-
	22	T/T	7.7	9.5	-	(1.2-3.0)	-
rs1325949	11	A/A	47.5	69.0	0.0035	0.00017	0.88
	12	A/G	44.3	22.4	1.8	2.5	-
	22	G/G	8.1	8.6	(1.2-2.6)	(1.5-3.9)	-
rs2421805	11	T/T	48.1	33.6	0.0014	0.012	0.0045
	12	T/G	44.4	48.7	0.58	0.55	0.37
	22	G/G	7.4	17.7	(0.4-0.8)	(0.3-0.9)	(0.2-0.8)
rs7543182	11	G/G	50.7	70.7	0.0096	0.00041	0.54
	12	G/T	42.5	20.7	1.67	2.34	-
	22	T/T	6.8	8.6	(1.1-2.5)	(1.5-3.8)	-
rs.7555874	11	G/G	50.7	69.8	0.014	0.00074	0.54
	12	G/A	42.5	21.6	1.62	2.25	-
	22	A/A	6.8	8.6	(1.1-2.4)	(1.4-3.6)	-
rs1409981	11	G/G	84.7	73.3	0.0021	0.012	0.040
	12	G/A	13.0	19.8	0.48	0.49	0.32
	22	A/A	2.3	6.9	(0.3-0.8)	(0.3-0.9)	(0.1-1.0)
rs.4147114	11	C/C	24.4	43.1	0.0012	0.00042	0.10
	12	C/G	53.4	42.2	1.72	2.34	-
	22	G/G	22.2	14.7	(1.2-2.4)	(1.5-3.8)	-
rs4147115	11	A/A	25.5	39.5	0.023	0.0098	0.34
	12	A/T	46.7	37.6	1.46	1.91	-
	22	T/T	27.8	22.9	(1.1-2.0)	(1.2-3.1)	-
rs4650093	11	C/C	51.4	65.5	0.092	0.013	0.44
	12	C/T	42.3	25.9	-	1.8	-
	22	T/T	6.4	8.6	-	(1.1-2.9)	-
rs17131478	11	G/G	61.6	74.6	0.035	0.018	0.79
	12	G/T	34.2	21.9	1.59	1.8	-
	22	T/T	4.1	3.5	(1.0-2.5)	(1.1-3.0)	-
rs17131479	11	C/C	62.2	75.0	0.039	0.018	0.91
	12	C/G	34.1	21.6	1.58	1.8	-
	22	G/G	3.7	3.4	(1.0-2.4)	(1.1-3.0)	-
rs7521005	11	A/A	51.6	65.5	0.10	0.014	0.44
	12	A/G	42.1	25.9	-	1.8	-
	22	G/G	6.3	8.6	-	(1.1-2.8)	-
rs7541092	11	G/G	62.4	74.8	0.040	0.023	0.77
	12	G/A	33.5	21.7	1.57	1.8	-
	22	A/A	4.1	3.5	(1.0-2.4)	(1.1-3.0)	-
rs1359835	11	G/G	88.6	79.1	0.0047	0.019	0.030
	12	G/C	10.9	17.4	0.45	0.49	0.13
	22	C/C	0.5	3.5	(0.3-0.8)	(0.3-0.9)	(0.01-1.1)
rs1327464	11	G/G	88.2	78.4	0.0043	0.017	0.031
	12	G/A	11.3	18.1	0.46	0.49	0.13
	22	A/A	0.5	3.4	(0.3-0.8)	(0.3-0.9)	(0.01-1.1)
rs1409161	11	G/G	30.8	25.9	0.040	0.35	0.014
	12	G/A	51.6	44.8	0.72	-	0.52
	22	A/A	17.6	29.3	(0.5-1.0)	-	(0.3-0.9)
rs34885906	11	T/T	85.5	94.0	0.026	0.021	0.0
	12	T/C	14.5	6.0	2.5	2.6	-
	22	C/C	0.0	0.0	(1.1-5.8)	(1.1-6.2)	-
rs2817864	11	T/T	53.4	61.2	0.056	0.17	0.021
	12	T/G	40.3	37.9	-	-	7.8
	22	G/G	6.3	0.9	-	-	(1.0-59.9)

[a]*P*-value for allele- or genotype frequency comparison between patients and controls using the chi-square test.

[b]OR, odds ratio.

[c]CI, confidence interval.

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in an exon as a silent SNP (sSNP), 4 (rs1325949, rs7543182, rs7555874, rs4147114) were in introns (iSNPs), and the remaining SNP (rs17131450) was a genome SNP[15]. We subsequently analyzed another 32 SNPs of *PTGER3 gene*. Therefore, we analyzed a total of 38 SNPs of *PTGER3* in 116 patient- and 221 control samples and we found that 20 SNPs were associated with SJS/TEN with mucosal MI (Table 2)[26].

We also examined the expression of EP3 (the protein of *PTGER3*) in normal human conjunctival epithelial cells because EP3 is constitutively expressed in mouse conjunctival epithelial cells[32]. RT-PCR assay showed that normal human conjunctival epithelial cells expressed *PTGER3* mRNA and immunohistochemistory confirmed the presence of EP3 protein[15, 33]. However, we did not find EP3 protein when we looked for the expression of EP3 in the conjunctival epithelium of SJS/TEN patients with MI although the protein was present in control conjunctival epithelium from patients with conjunctivochalasis (Fig. 3)[15, 33].

Our finding that compared to the controls, the expression of EP3 was greatly reduced in the conjunctival epithelium of patients with SJS/TEN with MI supports the genetic association between *EP3 gene* polymorphisms and SJS/TEN with MI and suggests that EP3 contributes functionally to the pathogenesis of SJS/TEN[15, 33].

Cold medicines including NSAIDs can inhibit the production of the EP3 ligand PGE_2. Because about 80% of our SJS/TEN patients with MI had used cold medications, possibly including NSAIDs, before disease onset, we posited that the observed *PTGER3* polymorphisms are associated with a cold medicine-related susceptibility to SJS/TEN with MI[15]. Our findings support the hypothesis that EP3 is involved in the development of SJS/TEN with MI.

SJS/TEN with MI and HLA

Earlier we reported that HLA-A*0206, absent in Caucasians, was strongly associated with SJS/TEN with MI in Japanese patients[14, 34]. An American ophthalmologists[35] reported that the HLA-B12 (HLA-Bw44) antigen was significantly increased in Caucasian SJS/TEN patients with MI and French dermatologists[36, 37] documented a significant increase in the HLA-B12 antigen in Caucasian SJS/TEN patients.

HLA-DQB1*0601 was reported to be associated in Caucasians with SJS with MI[38]. Our failure to detect this association in our patients[14] suggests strong ethnic differences in the HLA-SJS/TEN association.

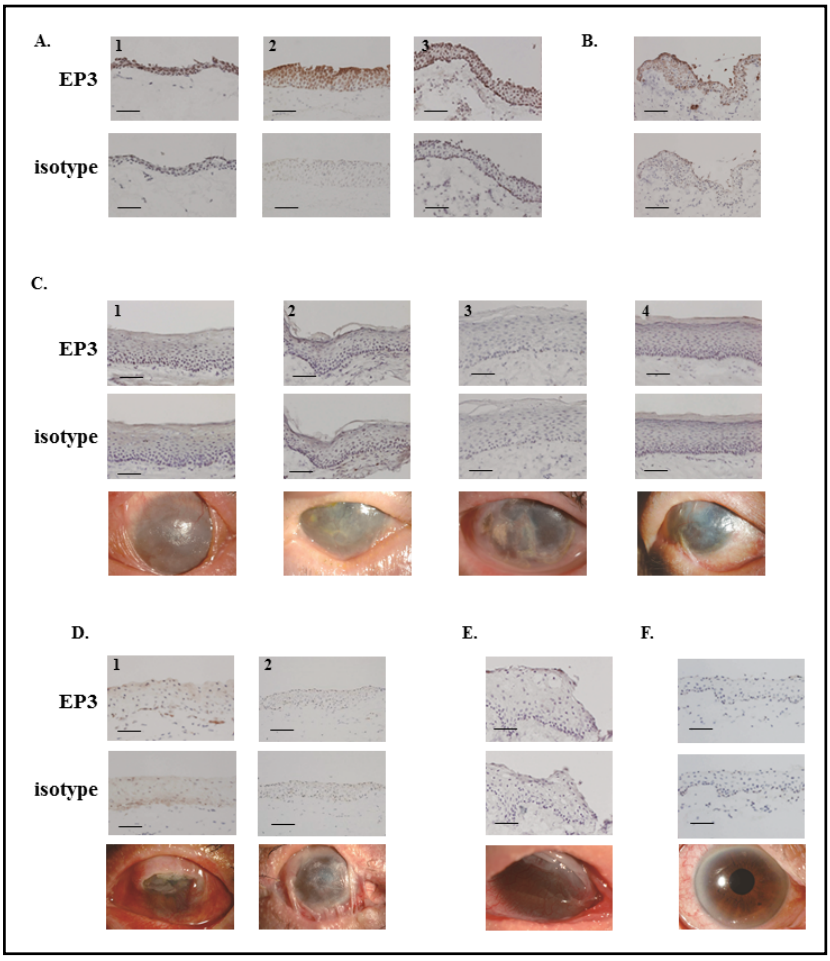

Fig. 3 Immunohistological analysis of prostaglandin E receptor subtype EP3 in the conjunctival epithelium of the controls and SJS/TEN patients with mucosal involvement

(A)Nearly normal conjunctival tissues from patients with conjunctivochalasis.
(B)Normal conjunctival tissue.
(C)Keratinized conjunctival tissues of SJS/TEN patients in the chronic stage.
(D)Non-keratinized conjunctival tissues of SJS/TEN patients in the sub-acute stage.
(E)Non-keratinized conjunctival tissues of SJS/TEN patients in the chronic stage.
(F)Visibly normal conjunctival tissue of an SJS/TEN patient with minor ocular sequelae (dry eye).
(C-F)The 3rd lane shows the ocular surface of SJS/TEN patients.
Each scale bar represents 100 μm
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With respect to the connection between drugs and severe cutaneous adverse reactions (SCAR) including SJS and TEN, there appears to be an association between the HLA-B*1502 allele[39] / HLA-A*3101[40] and carbamazepine-induced SCAR and between the HLA-B* 5801 allele and allopurinol-induced SCAR[41-43]. In carbamazepine-induced SCAR, the HLA-B*1502 allele showed a very strong association with carbamazepine-induced SJS/TEN in Han Chinese of Taiwan[39]. In the Japanese, the HLA-A*3101 allele exhibited a strong association with carbamazepine-induced SCAR including SJS/TEN and drug-induced hypersensitivity (DIHS)[40]. In allopurinol (a uric acid-lowering drug)-induced SCAR (including SJS, TEN, and DIHS) there was a strong association with HLA-B* 5801 in Han Chinese-[41], Caucasian-[42], and Japanese patients[43], suggesting a universal strong allopurinol-specific association between HLA-B*5801 and allopurinol-induced SCAR. However, allopurinol-induced SCAR may not elicit serious mucosal involvement at sites that include the ocular surface[44].

Epistatic interaction associated with SJS/TEN with MI

1)Interaction between the TLR3 and the EP3 gene

We showed that polymorphisms in *PTGER3*, the gene of EP3, a ligand of PGE_2, were significantly associated with SJS/TEN with MI[15] and that the PGE_2-EP3 pathway down-regulates the progression of murine experimental allergic conjunctivitis (EAC)[32]. We also documented that TLR3 polymorphisms are associated with SJS/TEN with MI[13], that the human ocular surface (corneal and conjunctival) epithelium strongly expresses TLR3, and that cytokine production is up-regulated by polyI:C, a TLR3 ligand in the ocular surface epithelium[18, 19, 45]. Based on these findings we examined the function of EP3 in polyI:C-stimulated primary human conjunctival epithelial cells using an EP3 agonist. We found that the EP3 agonist significantly suppressed the production and mRNA expression of CCL5, CXCL10, CXCL11, IL-6, TSLP, and MCP-1 in polyI:C-stimulated primary human ocular surface (corneal and conjunctival) epithelial cells. This suggests

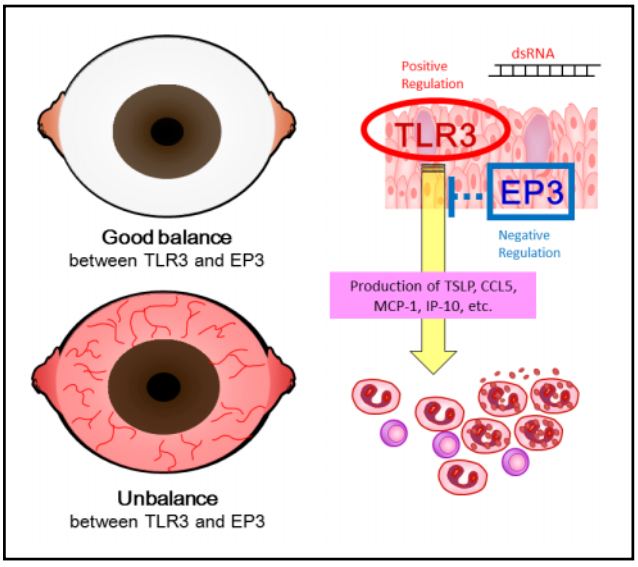

Fig. 4 Lack of balance between TLR3 and EP3 might trigger ocular surface inflammation
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that cytokine production by ocular surface epithelial cells in response to polyI:C stimulation can be suppressed via the activation of EP3[46-49].

In the past decade, SNPs were widely used as genetic markers for identifying human disease-susceptibility genes. It is now known that besides major single-locus effects, gene-gene interactions must also be considered[50]. In particular, non-additive (epistatic) models for some complex diseases fit with actual observations, suggesting interactions involving multiple loci[51]. We found a variable with susceptible effects on SJS/TEN with MI; these effects were involved in locus-pairs of *PTGER3-TLR3*. The *PTGER3* rs.4147114G/C- and the *TLR3* rs.3775296T/T SNPs exhibited a higher odds ratio (OR: 25.3, $p = 0.0000527$) than did the *TLR3* rs.3775296T/T SNP (OR: 5.35, $p = 0.00025$) or the *PTGER3* rs.4147114G/C SNP (OR: 2.66, $p = 0.0023$) alone[26].

Elsewhere we reported that compared to wild-type mice, conjunctival eosinophilic infiltration in EAC was significantly more marked in EP3-KO mice[32] and significantly less marked in TLR3-KO mice[52]. In EP3/TLR3-DKO mice the number of eosinophils in the lamina propria mucosae of the conjunctiva was decreased to a level similar to that in TLR3-KO mice; it was significantly lower than in EP3-KO- and wild-type mice[26]. Therefore, our findings suggest that in EAC, EP3 negatively regulates the eosinophilic infiltration induced by TLR3[26].

Our studies provide evidence of functional interactions between TLR3 and EP3 that might exert susceptibility effects with respect to SJS/TEN with MI and that the interactions are epistatic[26]. Consequently, we strongly suspect that an imbalance (a lack of balance) between TLR3 and EP3 can trigger mucosal inflammation at sites such as the ocular surface (Fig. 4).

Gene-Gene Interaction between HLA-A*0206 and TLR3

We also examined the multiplicative interaction(s) between HLA-A*0206 and 7 TLR3 SNPs in patients with SJS/TEN with MI. To study these interactions we analyzed the genotypes of HLA-A and 7 TLR3 SNPs in 110 Japanese SJS/TEN patients with severe MI and 206 healthy volunteers. We found that HLA-A*0206 exhibited a high odds ratio for SJS/TEN with MI (carrier frequency: OR=5.1; gene frequency: OR=4.0) and that there was a strong association with TLR3 rs.5743312T/T SNP (OR=7.4), TLR3 rs.3775296T/T SNP (OR=5.8), TLR3 rs.6822014G/G SNP (OR=4.8), TLR3 rs.3775290A/A SNP (OR=2.9), TLR3 rs.7668666A/A SNP (OR=2.7), TLR3 rs.4861699G/G SNP (OR=2.3), and TLR3 rs.11732384G/G SNP (OR=1.9)[27]. Our findings also suggest that the HLA-A*0206 - TLR3 SNP rs3775296T/T pair, which exhibited strong linkage disequilibrium (LD) with TLR3 rs.5743312, exerted more than additive effects (OR=47.7), and that the other pair, i.e. HLA-A*0206 - TLR3 rs.3775290A/A SNP (OR=11.4) which was in strong LD with the TLR3 rs7668666A/A, and HLA-A*0206 - the TLR3 rs4861699G/G SNP (OR=7.6) exerted additive effects that were stronger than the interactions within the TLR3 gene alone[27] (Fig. 5). These observations suggest that multiplicative interactions of HLA-A and the TLR3 gene may be required for the onset of SJS/TEN with MI.

Summary and Future Direction

In summary, not only environmental- but also genetic factors, including epistatic interactions, might play a role in an integrated etiology of SJS/TEN with MI. Since SJS/TEN with MI is very rare and probably has a complex genetic background, it is reasonable to posit that multiplicative interactions of genes are required for its phenotypic manifestation.

We also suggest that the pathogenesis of SJS/TEN with MI is associated with innate immune reaction abnormalities, especially those related with the epistatic interactions between TLR3 and EP3. Focusing on the innate immunity of the ocular surface might help to elucidate the pathogenesis

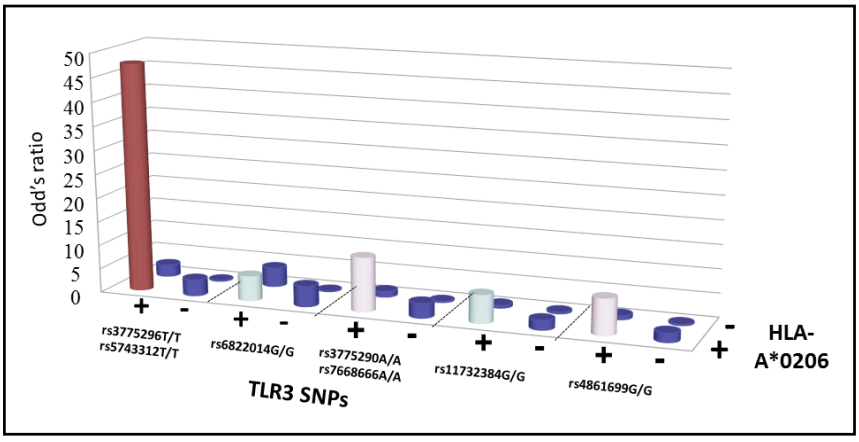

Fig. 5 Gene-gene interaction between HLA-A*0206 and TLR3

Interaction analysis suggested that the HLA-A*0206 - TLR3 SNP rs3775296T/T pair, which exhibited strong LD with TLR3 rs.5743312, exerted more than additive effects (OR=47.7), and that the other pair, HLA-A*0206 - TLR3 rs.3775290A/A SNP (OR=11.4) which was in strong LD with TLR3 rs7668666A/A- and TLR3 rs4861699G/G SNP (OR=7.6) exerted additive effects.

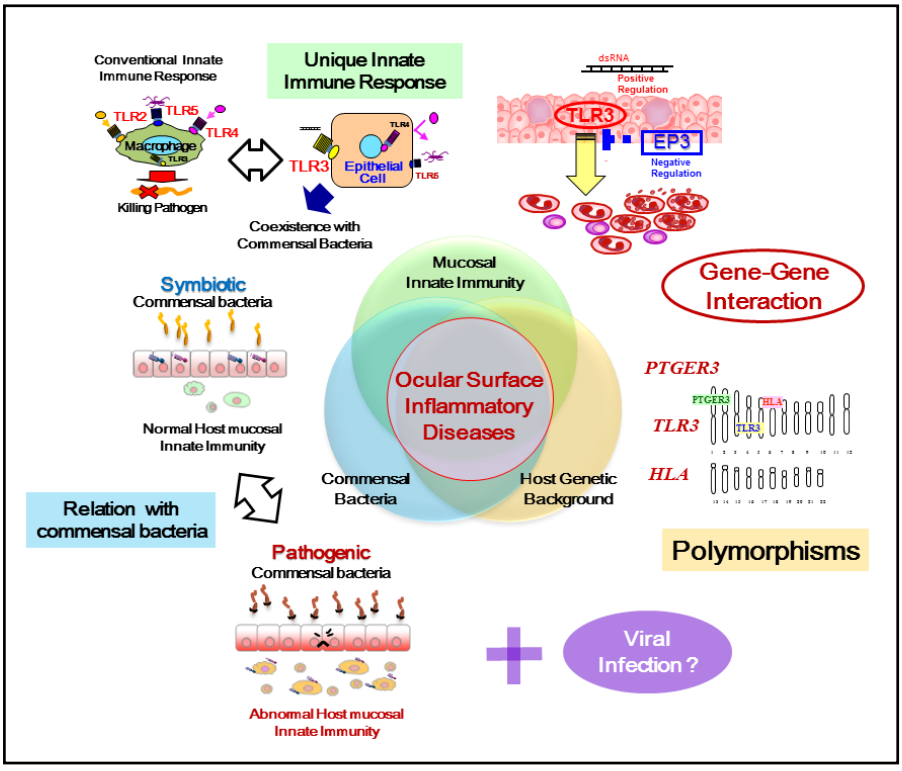

Fig. 6 Suggested pathophysiological mechanism of SJS/TEN with severe mucosal involvement

Ocular surface inflammatory diseases such as SJS/TEN with severe mucosal involvement might involve mucosal innate immunity, commensal bacteria, and a host genetic background.

The unique innate immune response of the ocular surface contributes to the coexistence with commensal bacteria. The pathogenicity of commensal bacteria is affected by anomalies in host mucosal innate immunity. The host genetic background such as polymorphisms is involved with host mucosal innate immunity. Gene-gene interactions also contribute to the pathobiological mechanisms of human ocular surface inflammatory diseases such as SJS/TEN with severe mucosal involvement.

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of ocular surface inflammatory diseases such as SJS/TEN with MI (Fig. 6).

It might be possible that the prophylaxis of SJS/TEN with MI will be created with prediction of the onset of the disease using these genetic predispositions. Moreover, the elucidate of the genetic predisposition might lead to reveal the pathological mechanism of SJS/TEN with MI and, furthermore, lead to establish the new treatment based on the pathological mechanism.

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Conflicts of interest

None

References

1) Stevens AM, Johnson FC: A new eruptive fever associated with stomatitis and opthalmia: report of two cases in children. Am J Dis Child. 1922; 24: 526-533.

2) Leaute-Labreze C, Lamireau T, Chawki D, Maleville J, Taieb A: Diagnosis, classification, and management of erythema multiforme and Stevens-Johnson syndrome. Arch Dis Child. 2000; 83: 347-352.
3) Forman R, Koren G, Shear NH: Erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. Drug Saf. 2002; 25: 965-972.
4) Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern RS, Anderson T, et al: Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. N Engl J Med. 1995; 333: 1600-1607.
5) Auquier-Dunant A, Mockenhaupt M, Naldi L, Correia O, Schroder W, Roujeau JC: Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. Arch Dermatol. 2002; 138: 1019-1024.
6) Yetiv JZ, Bianchine JR, Owen JA Jr: Etiologic factors of the Stevens-Johnson syndrome. South Med J. 1980; 73: 599-602.
7) Power WJ, Ghoraishi M, Merayo-Lloves J, Neves RA, Foster CS: Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. Ophthalmology. 1995; 102: 1669-1676.
8) Yamane Y, Aihara M, Ikezawa Z: Analysis of Stevens-Johnson syndrome and toxic epidermal necrolysis in Japan from 2000 to 2006. Allergol Int. 2007; 56: 419-425.
9) Sotozono C, Ang LP, Koizumi N, Higashihara H, Ueta M, Inatomi T, et al: New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology. 2007; 114: 1294-1302.
10) Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S: Association of IL4R polymorphisms with Stevens-Johnson syndrome. J Allergy Clin Immunol. 2007; 120: 1457-1459.
11) Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S: Association of Fas Ligand gene polymorphism with Stevens-Johnson syndrome. Br J Ophthalmol. 2008; 92: 989-991.
12) Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S: Association of combined IL-13/IL-4R signaling pathway gene polymorphism with Stevens-Johnson syndrome accompanied by ocular surface complications. Invest Ophthalmol Vis Sci. 2008; 49: 1809-1813.
13) Ueta M, Sotozono C, Inatomi T, Kojima K, Tashiro K, Hamuro J, et al: Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens-Johnson syndrome. Br J Ophthalmol. 2007; 91: 962-965.
14) Ueta M, Tokunaga K, Sotozono C, Inatomi T, Yabe T, Matsushita M, et al: HLA class I and II gene polymorphisms in Stevens-Johnson syndrome with ocular complications in Japanese. Mol Vis. 2008; 14: 550-555.
15) Ueta M, Sotozono C, Nakano M, Taniguchi T, Yagi T, Tokuda Y, et al. Association between prostaglandin E receptor 3 polymorphisms and Stevens-Johnson syndrome identified by means of a genome-wide association study. J Allergy Clin Immunol. 2010; 126: 1218-1225.
16) Sotozono C, Inagaki K, Fujita A, Koizumi N, Sano Y, Inatomi T, et al: Methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus epidermidis infections in the cornea. Cornea. 2002; 21: S94-S101.
17) Ueta M: Innate Immunity of the Ocular Surface and Ocular Surface Inflammatory Disorders. Cornea. 2008; 27: S31-S40.
18) Ueta M, Kinoshita S: Innate immunity of the ocular surface. Brain Res Bull. 2010; 81: 219-228.
19) Ueta M, Kinoshita S: Ocular surface inflammation is regulated by innate immunity. Prog Retin Eye Res. 2012; 31: 551-575.
20) Cho JH: The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol. 2008; 8: 458-466.
21) Ueta M, Hamuro J, Yamamoto M, Kaseda K, Akira S, Kinoshita S: Spontaneous ocular surface inflammation and goblet cell disappearance in I kappa B zeta gene-disrupted mice. Invest Ophthalmol Vis Sci. 2005; 46: 579-588.
22) Ueta M, Hamuro J, Ueda E, Katoh N, Yamamoto M, Takeda K, et al: Stat6-independent tissue inflammation occurs selectively on the ocular surface and perioral skin of IkappaBzeta-/- mice. Invest Ophthalmol Vis Sci. 2008; 49: 3387-3394.
23) Ohji M, Ohmi G, Kiritoshi A, Kinoshita S: Goblet cell density in thermal and chemical injuries. Arch Ophthalmol. 1987; 105: 1686-1688.
24) Sotozono C, Ueta M, Koizumi N, Inatomi T, Shirakata

Y, Ikezawa Z, et al: Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. Ophthalmology. 2009; 116: 685-690.
25) Yamamoto M, Yamazaki S, Uematsu S, Sato S, Hemmi H, Hoshino K, et al: Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta. Nature. 2004; 430: 218-222.
26) Ueta M, Tamiya G, Tokunaga K, Sotozono C, Ueki M, Sawai H, et al: Epistatic interaction between Toll-like receptor 3 (TLR3) and prostaglandin E receptor 3 (PTGER3) genes. J Allergy Clin Immunol. 2012; 129: 1413-1416.
27) Ueta M, Tokunaga K, Sotozono C, Sawai H, Tamiya G, Inatomi T, et al: HLA-A*0206 with TLR3 polymorphisms exerts more than additive effects in Stevens-Johnson syndrome with severe ocular surface complications. PLoS One. 2012; 7: e43650.
28) Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK: The R576 IL-4 receptor alpha allele correlates with asthma severity. J Allergy Clin Immunol. 1999; 104: 1008-1014.
29) Oiso N, Fukai K, Ishii M: Interleukin 4 receptor alpha chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan. Br J Dermatol. 2000; 142: 1003-1006.
30) Mitsuyasu H, Yanagihara Y, Mao XQ, Gao PS, Arinobu Y, Ihara K, et al: Cutting edge: dominant effect of Ile50Val variant of the human IL-4 receptor alpha-chain in IgE synthesis. J Immunol. 1999; 162: 1227-1231.
31) Howard TD, Koppelman GH, Xu J, Zheng SL, Postma DS, Meyers DA, et al: Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. Am J Hum Genet. 2002; 70: 230-236.
32) Ueta M, Matsuoka T, Narumiya S, Kinoshita S: Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. J Allergy Clin Immunol. 2009; 123: 466-471.
33) Ueta M, Sotozono C, Yokoi N, Inatomi T, Kinoshita S: Prostaglandin E receptor subtype EP3 expression in human conjunctival epithelium and its changes in various ocular surface disorders. PLoS One. 2011; 6: e25209.
34) Ueta M, Sotozono C, Tokunaga K, Yabe T, Kinoshita S: Strong Association Between HLA-A*0206 and Stevens-Johnson Syndrome in the Japanese. Am J Ophthalmol. 2007; 143: 367-368.
35) Mondino BJ, Brown SI, Biglan AW: HLA antigens in Stevens-Johnson syndrome with ocular involvement. Arch Ophthalmol. 1982; 100: 1453-1454.
36) Roujeau JC, Bracq C, Huyn NT, Chaussalet E, Raffin C, Duedari N: HLA phenotypes and bullous cutaneous reactions to drugs. Tissue Antigens. 1986; 28: 251-254.
37) Roujeau JC, Huynh TN, Bracq C, Guillaume JC, Revuz J, Touraine R: Genetic susceptibility to toxic epidermal necrolysis. Arch Dermatol. 1987; 123: 1171-1173.
38) Power WJ, Saidman SL, Zhang DS, Vamvakas EC, Merayo-Lloves JM, Kaufman AH, et al: HLA typing in patients with ocular manifestations of Stevens-Johnson syndrome. Ophthalmology. 1996; 103: 1406-1409.
39) Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, et al: Medical genetics: a marker for Stevens-Johnson syndrome. Nature. 2004; 428: 486.
40) Ozeki T, Mushiroda T, Yowang A, Takahashi A, Kubo M, Shirakata Y, et al: Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. Hum Mol Genet. 2011; 20: 1034-1041.
41) Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, et al: HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proc Natl Acad Sci U S A. 2005; 102: 4134-4139.
42) Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, et al: A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. Pharmacogenet Genomics. 2008; 18: 99-107.
43) Tohkin M, Kaniwa N, Saito Y, Sugiyama E, Kurose K, Nishikawa J, et al: A whole-genome association study of major determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. Pharmacogenomics J. 2013; 13: 60-69.
44) Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, et al: HLA-B*1511 is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. Epilepsia. 2010; 51: 2461-2465.
45) Ueta M, Hamuro J, Kiyono H, Kinoshita S: Triggering of TLR3 by polyI:C in human corneal epithelial cells to in-

duce inflammatory cytokines. Biochem Biophys Res Commun. 2005; 331: 285-294.
46) Ueta M, Matsuoka T, Sotozono C, Kinoshita S: Prostaglandin E2 suppresses poly I: C-stimulated cytokine production via EP2 and EP3 in immortalized human corneal epithelial cells. Cornea. 2012; 31: 1294-1298.
47) Ueta M, Matsuoka T, Yokoi N, Kinoshita S: Prostaglandin E2 suppresses polyinosine-polycytidylic acid (polyI: C)-stimulated cytokine production via prostaglandin E2 receptor (EP) 2 and 3 in human conjunctival epithelial cells. Br J Ophthalmol. 2011; 95: 859-863.
48) Ueta M, Matsuoka T, Yokoi N, Kinoshita S: Prostaglandin E receptor subtype EP3 downregulates TSLP expression in human conjunctival epithelium. Br J Ophthalmol. 2011; 95: 742-743.
49) Ueta M, Sotozono C, Yokoi N, Kinoshita S: Downregulation of monocyte chemoattractant protein 1 expression by prostaglandin E(2) in human ocular surface epithelium. Arch Ophthalmol. 2012; 130: 249-251.
50) Cordell HJ: Detecting gene-gene interactions that underlie human diseases. Nat Rev Genet. 2009; 10: 392-404.
51) Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, et al: Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet. 2001; 69: 138-147.
52) Ueta M, Uematsu S, Akira S, Kinoshita S: Toll-like receptor 3 enhances late-phase reaction of experimental allergic conjunctivitis. J Allergy Clin Immunol. 2009; 123: 1187-1189.