Table 4 | Results of association analyses using combined SJS/TEN patients' data

a. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	CM-SJS/TEN with SOC (Group 1a and Group 2a)	Control (Combined healthy controls)	p	Odds ratio (95% CI)
*A*02:06*	71/151 (47.0%)	87/639 (13.6%)	2.72E-20	5.63 (3.81–8.33)
*B*44:03*	39/151 (25.8%)	95/639 (14.9%)	0.00125	1.99 (1.30–3.05)

b. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and without SOC (Group 2c)

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	CM-SJS/TEN with SOC (Group 1a and Group 2a)	CM-SJS/TEN without SOC (Group 2c)	p	Odds ratio (95% CI)
*A*02:06*	71/151 (47%)	2/16 (12.5%)	0.00812	6.21 (1.36–28.28)
*B*44:03*	39/151 (25.8%)	0/16 (0%)	0.02023	11.59* (0.68–197.7)

c. Comparison of CM unrelated SJS/TEN with SOC and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis
HLA genotype	CM unrelated-SJS/TEN with SOC (Group 1d and Group 2d)	Control (Combined healthy controls)	p
*A*02:06*	7/52 (13.5%)	87/639 (13.6%)	0.975
*B*44:03*	6/52 (11.5%)	95/639 (14.9%)	0.514

d. Comparison between Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b) and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b)	Control (Combined healthy controls)	p	Odds ratio (95% CI)
*A*02:06*	37/73 (50.7%)	87/639 (13.6%)	2.54E-15	6.52 (3.91–10.88)
*B*44:03*	20/73 (27.4%)	95/639 (14.9%)	0.0059	2.16 (1.27–3.78)

*Woolf's correction.
P: P values obtained by χ^2-tests.
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: Confidence interval.

patients with AR-SJS/TEN with SOC, we found a significant association with both alleles (*HLA-A*02:06*, p = 2.5 × 10^{-15}, OR = 6.5; *HLA-B*44:03*, p = 0.0059, OR = 2.2) (Table 4d).

Discussion

In this study we examined possible HLA risk factors for CM-SJS/TEN with SOC using two independently collected data sets of Japanese SJS/TEN patients.

The carrier frequency of *HLA-A*02:06*, which we reported to have a very strong association with causative drug-unspecified SJS/TEN with SOC[15,19], was significantly higher in CM-SJS/TEN with SOC patients than in the healthy controls. This significant association was maintained in AR-SJS/TEN with SOC.

On the other hand, the carrier frequency of *HLA-A*02:06* in the 16 CM-SJS/TEN without SOC patients of Group 2c and the 52 CM-unrelated SJS/TEN with SOC patients from Groups 1d and 2d did not significantly differ from that in our healthy controls. These results suggest that *HLA-A*02:06* is a risk factor for CM-SJS/TEN with SOC but not for CM-SJS/TEN without SOC or CM-unrelated SJS/TEN with SOC.

Moreover, *HLA-A*02:06* and *HLA-B*44:03* might not be primarily associated with only infection related SJS/TEN, because drug-unrelated SJS/TEN with SOC in KPUM, which seemed to be only infectious agents-related SJS/TEN, was not associated with *HLA-A*02:06* and *HLA-B*44:03* in our preliminary study (Supplemental Table 1).

The carrier frequeny of *HLA-A*02:06* in all of our healthy controls was 13.6% (Tables 2 and 3), indicating that *HLA-A*02:06* is a very common allele in the Japanese. However, as it is very rare in Caucasians and less frequent in Southern Han Chinese[20], in these populations, this allele might not be a major risk factor for CM-SJS/TEN with SOC. We also found a significant association between *HLA-B*44:03* and CM-SJS/TEN with SOC (including AR-SJS/TEN with SOC). This association was not detected in CM-SJS/TEN without SOC patients nor in CM-unrelated SJS/TEN with SOC patients. This again suggests *HLA-B*44:03* as a risk factor for CM-SJS/TEN with SOC. Data on our controls (Tables 2 and 3) indicate that *HLA-B*44:03* is a common *HLA-B* type in the Japanese population. Unlike *HLA-A*02:06*, *HLA-B*44:03* is observed in Asians, Caucasians and Africans[21]. Reports from the USA[22] and France[23,24] showed that the *HLA-B12* (*HLA-Bw44*) antigen was significantly increased in Caucasian SJS patients. The *HLA-B12* antigen is mainly coded by *HLA-B*44:02* or *HLA-B*44:03* (http://www.allelefrequencies.net/).

Cold medicines were reported to be major causative drugs in SJS/TEN in Europe[4] and in its drug safety communications, the U.S. Food and Drug Administration (http://www.fda.gov/Drugs/DrugSafety/ucm363041.htm) alerted to the possibility of serious skin reactions to acetaminophen. The significant association of HLA-B12 with SJS/TEN in European patients may be attributable to their genetic backgrounds. To determine whether *HLA-B*44:03* is a common risk

factor for CM-SJS/TEN with SOC in various populations, independent association studies in divergent ethnic groups are needed.

Because *HLA-A*02:06* is rarely a haplotype with *HLA-B*44:03* (http://www.allelefrequencies.net/), these two HLA alleles might be independent genetic risk factors that render the host susceptible to severe mucosal disorders and to severe sequelae such as visual disturbance when SJS/TEN develops after the administration of cold medicines including NSAIDs. In our study, 96 of 151 patients (63.6%) with CM-SJS/TEN with SOC (group 1, n = 131; group 2, n = 20) harbored either *HLA-A*02:06* or *HLA-B*44:03*. On the other hand, only 177 of our 639 controls (27.7%) had one of these *HLA* alleles.

Forman et al.[25] and Leaute-Labreze[26] reported other infectious agents as triggers of SJS/TEN. Elsewhere[27] we showed that rs3775296T/T, a SNP of *Toll-like receptor 3 (TLR3)*, was a risk factor for SJS/TEN with SOC and that the interaction between rs3775296T/T and *HLA-A*02:06* exerted more than additive effects. TLR3 is a pattern-recognizing receptor related to innate immunity after viral infections that often produce common cold symptoms. Moreover, cold medicines such as acetaminophen and NSAIDs, including ibuprofen and loxoprofen, commonly down-regulate the production of prostanoid including PGE_2. We also reported earlier that in our study population, EP3, which is one of the PGE_2 receptors, polymorphisms were strongly associated with SJS/TEN with SOC[14] and that the EP3 protein levels were much lower in the conjunctival epithelial cells of SJS/TEN patients than in the control subjects[14,28]. It is noteworthy that in our earlier study of SJS/TEN with SOC patients[14] about 80% had CM-SJS/TEN with SOC. It might be possible that not only cold medicine but cold medicine with infectious agent could cause CM-SJS/TEN with SOC, because the patients develop CM- SJS/TEN with SOC by taking cold medicines after having common cold induced by infectious agents. We believe that interactions between *HLA* risk factors detected in the current study and *TLR3*, and/or *EP3* might be keys in the pathogenesis of CM-SJS/TEN with SOC.

In summary, we reported the association between certain *HLA* types and CM-SJS/TEN with SOC. We propose that *HLA-A*02:06* and *HLA-B*44:03* be considered as strong risk factors for CM-SJS/TEN with SOC. Our findings may help to elucidate the pathogenesis of CM-SJS/TEN with SOC.

Methods

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

Patients and controls. Japanese SJS/TEN patients (n = 236) were independently recruited at Kyoto Prefectural University of Medicine (KPUM)(Group 1, n = 162) and by the Japan Severe Adverse Reactions Research Group, mainly conducted by the National Institute of Health Sciences (NIHS) (Group 2, n = 74).

Between October 2004 and May 2013, 162 SJS/TEN with SOC were treated at Kyoto Prefectural University of Medicine; of these, 71 were included in our previous study[15]. The diagnosis of SJS/TEN with SOC was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and the involvement of at least 2 mucosal sites including the oral cavity and ocular surface. Some of the patients had developed SJS/TEN many years before recruitment for this study. Of the 162 patients in Group 1, 131 patients had taken cold medicines such as NSAIDs and multi-ingredient cold medications for a few ~ several days before disease onset for common-cold symptoms; they were classified as CM-SJS/TEN with SOC (Group 1a). Although the specific drugs were not identified by all 131 CM-SJS/TEN with SOC patients, 59 of 131 CM-SJS/TEN with SOC patients (45%) reported taking medicines containing acetaminophen (AR-SJS/TEN with SOC, Group 1b). Among the 162 of SJS/TEN with SOC patients (Group 1), 14 patients (Group 1d) were classified as CM unrelated-SJS/TEN with SOC, because they manifested anticonvulsants-related SJS/TEN with SOC (n = 10) or SJS/TEN with SOC after being treated with antimalarial-, anticancer-, or anti-depressive agents or steroids n = 4). We also excluded 17 patients; in 9 SJS/TEN with SOC the drugs were unknown and in 8 SJS/TEN with SOC were not related to drugs.

Group 2 (n = 74) consisted of patients with newly-developed SJS/TEN; they were recruited between June 2006 and May 2013 by participating institutes or via a nationwide blood sampling network operated by the NIHS in cooperation with the Ministry of Health, Labour and Welfare, the Pharmaceutical and Medical Devices Agency, and the Federation of Pharmaceutical Manufacturers' Association of Japan. The criteria proposed by Bastuji-Garin et al.[16] were used for a diagnosis of SJS/TEN in this group.

Ocular surface complications were judged to be severe ocular complications (SOC) when pseudo-membrane formation and/or conjunctival or corneal epithelial defects were observed in the acute phase. As shown in Table 1, Group 2 (n = 74) consisted of 20 patients with CM-SJS/TEN with SOC (Group 2a), all but 6 of these presented with AR-SJS/TEN with SOC (Group 2b). Group 2 also included 16 patients with CM-SJS/TEN without SOC (Group 2c), and 38 patients with CM-unrelated-SJS/TEN with SOC (Group 2d). The background of the 236 patients with SJS/TEN in group1 and group2 is summarized in Table 1.

Healthy Japanese volunteers (n = 639) served as the controls. They were independently recruited by the University of Tokyo (n = 419)[17] and by Kyoto Prefectural University of Medicine (n = 220)[18] and served for comparison studies of patient groups 1 and 2, respectively. In this study we enrolled only mainland Japanese.

HLA genotyping. We analyzed *HLA-A, -B, and -C* of all 162 group 1 patients, which consist of 131 CM-SJS/TEN with SOC (group 1a), 14 CM-unrelated (other medicine related) SJS/TEN with SOC (group 1d), and 17 SJS/TEN with SOC excluded because of being drug-unrelated and detail unknown. We performed polymerase chain reaction (PCR) assays followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial bead-based typing kits (Wakunaga, Hiroshima, Japan). In group 2 (n = 74) we performed high-resolution HLA typing with a sequence-based method using SeCoreA, -B, and -C, locus sequencing kits (Invitrogen Corp., Brown Deer, WI, USA) and ABI 3730 and 3130 DNA sequencers (Applied Biosystems, Foster City, CA, USA). HLA genotypes were assigned using Assign SBT- or Assign ATF software (versions 3.2.7b and 1.0.2.41; respectively, Conexio Genomics, Western Australia, Australia). We also genotyped all volunteers for *HLA-A, -B, and -C* using PCR-SSO and commercial bead-based typing kits (Wakunaga or One Lambda, CA, USA).

Statistical analysis. We compared the carrier frequency of individual HLA alleles between our patients and controls based on the dominant model using the χ^2-test (Labo Server software;World Fusion, Tokyo, Japan) or Fisher's exact test (JMP version 7.0.1 software; SAS Institute Japan Ltd., Tokyo, Japan). Significance levels were corrected with the Bonferroni correction for multiple comparisons.

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Author contributions

M.U., N.K. and K.T. wrote the main manuscript text and made Table, M.U., N.K., C.S., K.T., Y.S., H.S., H.M., E.S., K.M., R.N., M.N., M.A., K.M., Y.T., H.F., M.M., Z.I. and S.K. contributed to material of the research and reviewed the manuscript.

Additional information

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Upregulation of Toll-like receptor 5 expression in the conjunctival epithelium of various human ocular surface diseases

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ABSTRACT

Aims To examine the expression of Toll-like receptor (TLR) 5 in the conjunctival epithelium of patients with severe ocular surface diseases.

Methods Immunohistochemical study of TLR5 was performed on conjunctival tissues obtained from patients undergoing surgical reconstruction of the ocular surface to treat Stevens-Johnson syndrome (SJS) (n=4), ocular cicatricial pemphigoid (OCP) (n=3), chemical eye burn (n=3), and pterygium (n=2), and on nearly normal conjunctival tissues obtained during surgery for four cases of conjunctivochalasis as a control.

Results TLR5 protein was consistently and abundantly expressed in the conjunctival epithelium and detected only at the basal and wing cells. However, in the conjunctival epithelium obtained from the patients with SJS, OCP and chemical eye burns, the TLR5 protein was detected at not only the basal and wing cells but also at the superficial cells. TLR5 protein detected in the pterygium patients mirrored that detected in the controls.

Conclusions Although TLR5 was normally present on the basal and wing cells of conjunctival epithelium with spatially selective presence, it was expressed on not only the basal and wing cells but also the superficial cells in the conjunctival epithelium of patients with SJS, OCP or chemical eye burns, suggesting that TLR5 might be upregulated in the conjunctival epithelium of these diseases.

INTRODUCTION

The ocular surface epithelium serves as the defensive front line of the innate immune system, and Toll-like receptors (TLRs) are known to be one of the key receptors of the innate immune system. Reportedly, TLRs are pattern-recognition receptors that sense conserved pathogen-associated molecular patterns (PAMPs), and are the key receptors for the recognition of microbes.[1] TLRs are a type of transmembrane protein composed of three major domains, and are characterised by LRRs (leucine-rich repeats) in the ectodomain which mediate the recognition of their respective PAMPs. TLR5 recognises bacterial flagellin, a component protein of bacterial flagella.[2 3] Flagella are present in both gram-positive and gram-negative bacteria and are essential for bacterial motility, invasion and chemotaxis.[4] We previously reported that human ocular surface epithelial cells, both corneal and conjunctival, express TLR5-specific mRNA and TLR5 proteins, and TLR5 proteins were detected at basal and wing site cells.[5 6] The purpose of this present study was to examine the expression of TLR5 in the conjunctival epithelium of patients with severe ocular surface disorders.

METHODS

This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan, and all experiments were conducted in accordance with the tenets set forth in the Declaration of Helsinki. The purpose of the research and the experimental protocol were explained to all patients, and their informed consent was obtained before their involvement in the study.

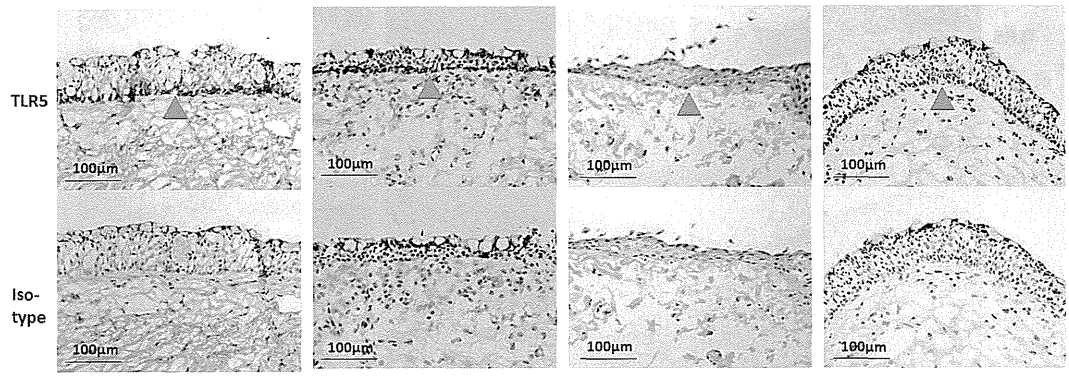

Figure 1 Immunohistological analysis of Toll-like receptor (TLR) 5 in the conjunctival epithelium of the controls. Localisation of TLR5 in human conjunctival epithelium as control tissue, from four conjunctivochalasis cases which underwent surgery. The conjunctival tissue was incubated with anti-TLR5 antibody. Isotype control incubation was used as the negative control.

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For the immunohistochemical study, the control samples were nearly normal human conjunctival tissues obtained at the time of surgery for the treatment of conjunctivochalasis (four cases). Conjunctivochalasis is a normal ageing related change, and it is an isolated bilateral condition in which redundant bulbar conjunctival tissue interposes between the globe and the lower eyelid.[7] Cicatricial conjunctival tissues were also obtained from patients undergoing surgical reconstruction of their ocular surface that had been devastated due to Stevens-Johnson syndrome (SJS) (four cases), ocular cicatricial pemphigoid (OCP) (three cases), and chemical eye burns (three cases). In addition, the conjunctival tissues of patients with pterygium (two cases) were obtained. Pterygium is reportedly a benign growth of the conjunctiva that is thought to be caused by exposure to ultraviolet light.[8]

For TLR5 staining, we used mouse antihuman TLR5 monoclonal antibody (mAb; Abcam, Cambridge, UK) (TLR5: TLR5 antibody (19D759.2)) in a 0.5 μg/μL dilution with blocking solution. As isotype controls, mouse IgG2a X0943 (Dako Cytomation, Kyoto, Japan) in a 0.1 μg/μL dilution was used. After incubation overnight at 4°C in a moist chamber, the sections were thoroughly washed with 0.01 M phosphate buffered saline. Next, the sections

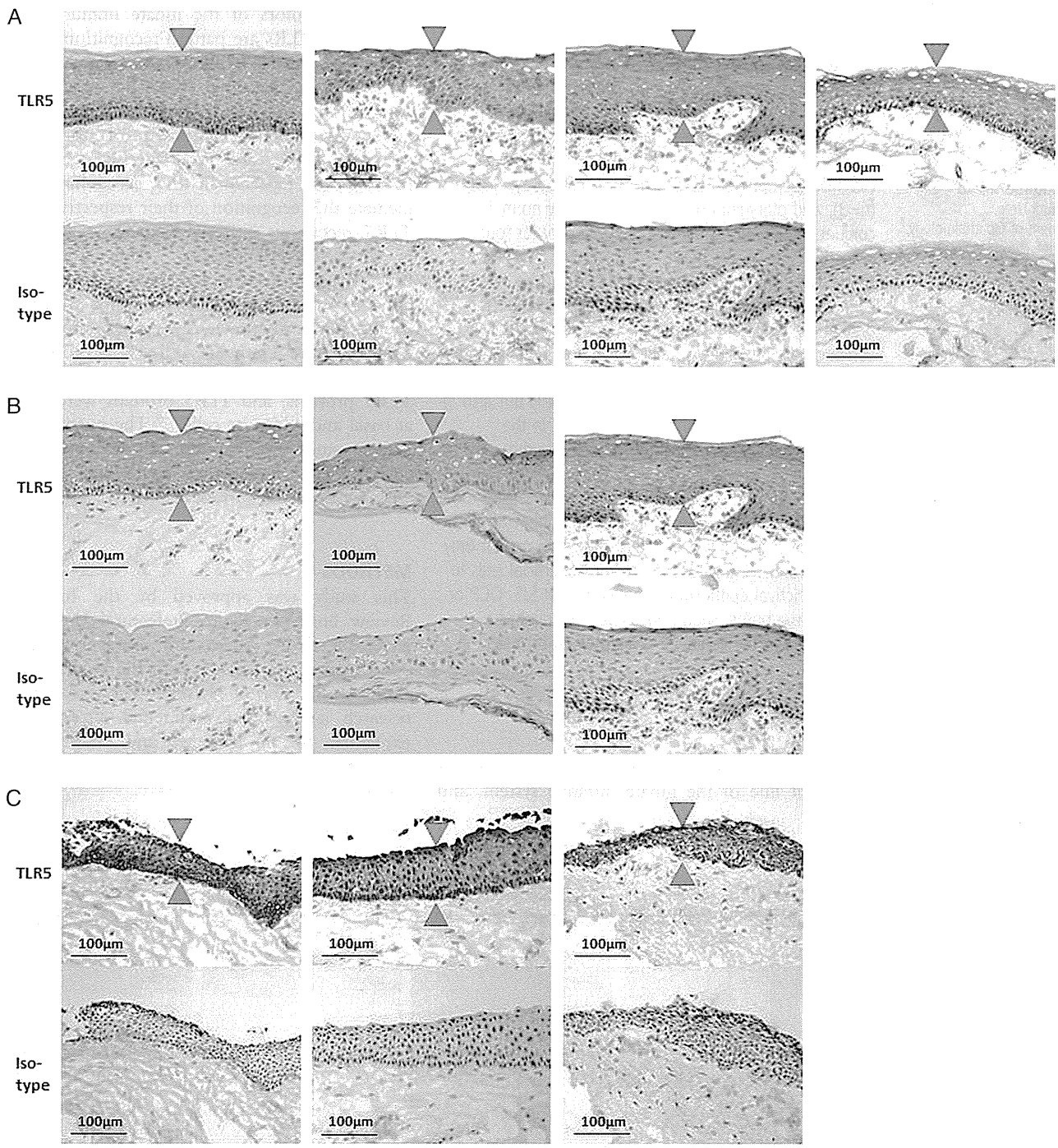

Figure 2 Immunohistological analysis of Toll-like receptor (TLR) 5 in the conjunctival epithelium of various ocular surface disorders. Localisation of TLR5 in the conjunctival epithelium of various conjunctival disorders; four Stevens-Johnson syndrome cases in the chronic stage (A), three ocular cicatricial pemphigoid cases (B), and three chemical burn cases (C). The conjunctival tissue was incubated with anti-TLR5 antibody. Isotype control incubation was used as the negative control.

were subjected to secondary antibody reactions with biotin conjugated donkey anti-mouse IgG Fab fragments in a 0.5 μg/μL dilution. Avidin and biotinylated horseradish peroxidase macromolecular complex reagent (VECTASTAIN ABC reagent; Vector Laboratories, Inc, Burlingame, California, USA) was then applied to the section for 30 min and 3,3′-diaminobenzidine peroxidase substrate solution (DAB substrate kit; Vector Laboratories) was added to the slide sections as a chromogenic substrate. Finally, the sections were counterstained using haematoxylin.

RESULTS

Conjunctival tissues obtained at the time of ocular surface reconstruction surgery were subjected to immunohistochemical study to determine the presence and localisation of TLR5 expression in stratified conjunctival epithelium. In human conjunctival epithelium of the control conjunctiva, TLR5 protein was consistently and abundantly expressed; however, it was detected only at the basal and wing cells (figure 1). These results are consistent with those of our previous report.[6] On the other hand, in the epithelium of the conjunctival tissues obtained from the four SJS patients (figure 2A), the three OCP patients (figure 2B), and the three patients being treated for chemical eye burns (figure 2C), the TLR5 protein was detected in all layers from the basal and wing cells to the superficial cells. However, in the epithelium of the conjunctival tissues obtained from the two pterygium patients (figure 3), the TLR5 protein was detected in its basal and wing cells, but not the superficial cells, the same as with the controls.

DISCUSSION

The findings of this study showed that in devastating ocular surface disorders such as SJS, OCP and chemical eye burns, TLR5 is expressed not only on the basal and wing cells but also on the superficial cells, although TLR5 is expressed on only the basal and wing cells in normal human conjunctival epithelium as we previously reported.[6] In the conjunctival epithelium obtained from the two pterygium patients, TLR5 protein was also consistently and abundantly expressed, yet detected only at the basal and wing cells, the same as with normal human conjunctival epithelium. The above findings suggest that TLR5 might be upregulated in the ocular surface of patients with these devastating ocular surface disorders.

It should be noted that previous reports have investigated the expression of TLR5 in other human disorders. Recent findings have revealed that both in rheumatoid arthritis (RA) and osteoarthritis, TLR5 immunostaining is significantly higher on synovial tissue lining, sublining macrophages and endothelial cells compared with controls.[9] The findings of that study also suggest that the expression of TLR5 is upregulated in RA disease progression, that RA is reportedly a chronic autoimmune disorder in which the innate immune system plays an important role, and that intestinal epithelium reportedly expresses TLR5.

Rhee *et al*[10] reported that the flagellin/TLR5 response is confined to the basal cells, not the superficial cells, of intestinal mucosa, indicating that the intact colonic mucosa is not responsive to luminal bacterial flagellin. We also reported that on ocular surface epithelium, TLR5 was expressed on only the basal and wing cells, not the superficial cells, thus suggesting that in the normal condition, the intact ocular surface could not be responsive to commensal bacterium of the ocular surface.[11] In a very recent study, it was reported that TLR5 mRNA tends to be upregulated in the active phase of ulcerative colitis (UC) compared to UC quiescent disease, and that there are positive correlations between TLR5 mRNA and endoscopic and histological activity.[12] Moreover, in irritable bowel syndrome (IBS) of dysfunctional colitis that partly results from low grade mucosal inflammation, it was reported that TLR5 mRNA was upregulated in colonic biopsies from active IBS patients.[13] Therefore, TLR5 expressions may be upregulated in the diseases associated with chronic inflammations or in an active inflammation phase.

In chronic phase SJS and OCP patients, it was reported that the number of neutrophil elastase-positive cells and CD4-positive cells was increased in the epithelium and substantia propria of the pannus tissue. In addition, CD3-positive cells in the substantia propria of the pannus were reportedly slightly increased in both OCP and SJS patients, yet other kinds of infiltrating cells were not increased.[14] Therefore, upregulation of TLR5 in the epithelium of the conjunctival tissues of SJS, OCP, and chemical eye burns might be involved in the chronic inflammation of these respective diseases. Although it has been reported that there are chronic inflammatory cells in pterygium,[15] the difference of the extent of TLR5 expression between pterygium and the devastating ocular surface disorders might suggest the different quality of the chronic inflammation.

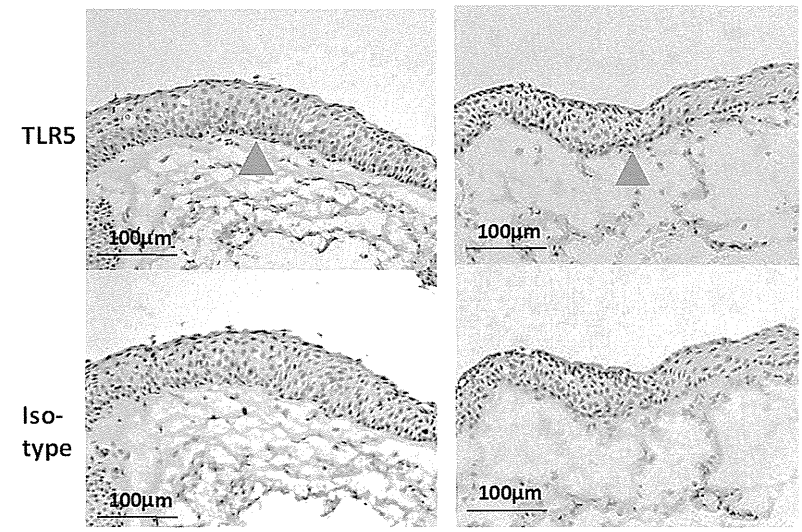

Figure 3 Immunohistological analysis of Toll-like receptor (TLR) 5 in the conjunctival epithelium obtained from two pterygium patients. Localisation of TLR5 in the conjunctival epithelium from the two pterygium cases which underwent surgery. The TLR5 protein was detected only at the basal and wing sites, the same as with the control. Isotype control incubation was used as the negative control.

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Upregulation of Toll-like receptor 5 expression in the conjunctival epithelium of various human ocular surface diseases

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角膜疾患関連続発緑内障への対処法

Therapeutic Strategy for the Secondary Glaucoma Related to Corneal Diseases

森　和彦*

はじめに

角膜疾患関連続発緑内障は難治緑内障の一つとされる．とくに角膜移植後に合併する緑内障は移植後の失明原因として常に上位に位置しており，角膜が透明になっても視神経障害のために失明に至る例も少なくない．術後の一過性高眼圧を除いた角膜移植後続発緑内障の発症頻度は全体では30%程度とされているが，角膜移植の原因疾患によってその頻度が大きく異なっている．たとえば無水晶体性水疱性角膜症では続発緑内障発症頻度が20～70%ともっとも高く，円錐角膜や先天性遺伝性角膜内皮ジストロフィ（congenital hereditary corneal endothelial dystrophy：CHED）などでは低いとされている[1]．一般に角膜移植後の続発緑内障発症・増悪のリスクとしては，角膜移植前から存在している緑内障の既往，無水晶体無硝子体眼，眼内レンズ（intraocular lens：IOL）摘出術併用例などがあげられている．

角膜疾患に関連した緑内障の眼圧上昇メカニズム（**表1**）は，角膜の病態や移植の有無，経過期間によって異なっており，異常な増殖組織や炎症に続発した隅角閉塞，移植後早期の前房内残留粘弾性物質，出血，炎症，角膜浮腫や浅前房による周辺虹彩前癒着（peripheral anterior synechia：PAS），瞳孔ブロック，ステロイド緑内障，拒絶反応に伴う炎症・虹彩前癒着の進行，悪性緑内障など，さまざまな原因があげられる．近年はシクロスポリンに代表されるステロイド以外の免疫抑制療法が広く用いられるようになり，ステロイド長期使用例の減少に伴いステロイド緑内障の頻度は減少傾向にある．いずれの病態においても角膜疾患続発緑内障では，高眼圧のみならず治療としての緑内障手術によっても惹起された炎症が角膜の透明性維持に悪影響を及ぼすため，十分な管理上の注意が必要である．

I　重要ポイント

1. 眼圧測定

角膜疾患関連続発緑内障の診断においてもっとも重要なポイントは眼圧上昇の判定である．眼圧測定のゴールデンスタンダードであるGoldmann圧平眼圧計（Goldmann applanation tonometer：GAT）は角膜厚や角膜表面性状の影響を受け，不整な眼表面状態や角膜厚が厚い水疱性角膜症では正確な眼圧が測定困難である．しかし，DSAEK（Descemet's stripping automated endothelial keratoplasty）やDMEK（Descemet's membrane endothelial keratoplasty）では角膜厚は大きくは変化しないため眼圧値に対する影響は少ない．眼表面に非接触で測定可能という利点から頻用されるノンコンタクトトノメーター（non-contact tonometer：NCT）は，角膜疾患例において安定した測定値を得ることは困難であり，あくまでも参考程度にしかならない．トノペン®（Reichert社）は接触面積が小さいことから眼表面疾患でも正確に測定できる可能性が高いが，実際にはばらつきが大きく，やはり参考程度の値しか得られない．リバウンドトノメーターであるアイケア®（ICARE FINLAND

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社）は接触面積がさらに小さいことからかなり正確な眼圧値が得られるが，GATと同様に角膜厚の影響を受けるので当てる場所によって値が大きく異なることがある．さらには培養角膜上皮移植や羊膜移植などの眼表面再建術の術後早期には角膜に触れることすら差し控えざるを得ない．最終的にはGATとNCT，その他の眼圧計の値を参考にしながら，眼瞼上から強膜越しに眼球に触れて判断するタクタイル法で眼圧上昇を確認するのがもっとも確実である．そのためにも常日頃から眼球を触診し，眼瞼上から眼圧を推測する感性を養っておく必要がある．

2. 眼圧上昇は一過性か持続性か

次に重要なポイントは原疾患の治療経過と眼圧上昇との関係である．たとえば角膜移植後早期には，前房内の残存粘弾性物質や炎症により眼圧が高値を示すことが多いが，これらは経過をみているうちに下降してくる一過性のものである．一方，移植後を含めた角膜疾患の経過中には点眼・内服を含めてステロイド薬を使用することが多く，眼圧上昇例の中には高頻度にステロイド緑内障が含まれている．したがって治療経過，とくにステロイド使用歴と眼圧上昇との時間的関係について知っておくことはきわめて重要である．通常，ステロイドレスポンダーはステロイド使用開始後，2週間程度経過してから眼圧が上昇してくることが多いが，線維柱帯からの房水流出に余裕のない場合にはステロイド開始後，短期間で眼圧上昇をきたすこともある．また，眼内炎症遷延時には炎症に伴って眼圧が上昇している場合もあり，炎症程度との関連性も眼圧上昇機序を理解するうえで重要となる．さらに症例によっては基礎疾患として原発緑内障や外傷性緑内障などを有している場合もあり，問診による既往歴の聴取も重要となる．

3. 隅 角 所 見

PASの有無は緑内障の治療法や予後に影響を与えるもっとも重要な因子であり，van Herick法によって隅角開大度を判定するのはもちろんのこと，眼表面状態が許されるなら隅角検査を実施すべきである．ただ角膜疾患の中には混濁のためにvan Herick法による隅角開大度判定すら困難な場合が多いし，移植片拒絶反応や移植した上皮細胞の脱落の恐れから基本的に接触型検査自体が不可な場合もある．このような場合でも緑内障手術の適応や術式を決定するためには隅角検査が必須であることから，接触面積の少ないZeiss型やSussman型の隅角鏡を用い，スコピゾルを大量に使用したり治療用ソフトコンタクトレンズ上から行ったりすることで何とか隅角検査を試みることが大切である．そのためにも常日頃から角膜上皮に負担をかけない隅角検査手技をマスターしておくことが必要である．

非接触式検査である前眼部光干渉断層計（optical coherence tomography：OCT）による画像解析は，隅角検査が不可能な症例の隅角形状を判断する際の次善の策として有用である．現在利用できる前眼部OCTの中では，タイムドメイン型のVisante™（Carl Zeiss社）と比較して，スペクトラルドメイン型のCasia®（トーメーコーポレーション）のほうが短時間かつ高解像度で全周の隅角をスキャンできるため有用性に勝る．ただ非接触式であるがゆえに機能的閉塞と器質的閉塞の差のような動的変化を捉えることは困難である．また，あくまでもカラー情報をもたず隅角形状を捉えるのみであることから，隅角の異常所見を捕捉する能力としては隅角検査に劣ることを認識すべきである．また，さらに後方の虹彩裏面や毛様体突起部などの所見を得るためには，超音波生体顕微鏡（ultrasound biomicroscope：UBM）が有用であるが，接触式検査であるために隅角検査と同様の制限がある．

4. 眼表面性状と炎症

点状表層角膜症や遷延性角膜上皮欠損の有無，涙液動態などの眼表面性状は，緑内障点眼治療における薬剤選択に影響する因子として重要であるため，フルオレセイン染色を用いてしっかりと確認する必要がある．また，通常は見落とされがちな上方もしくは下方結膜の状態に関しても，緑内障の術式選択に当たって濾過手術やチューブシャント手術を成功させるために非常に重要な所見であり，瘢痕性変化や瞼球癒着，結膜嚢短縮の有無などについて十分な診察が必要である．