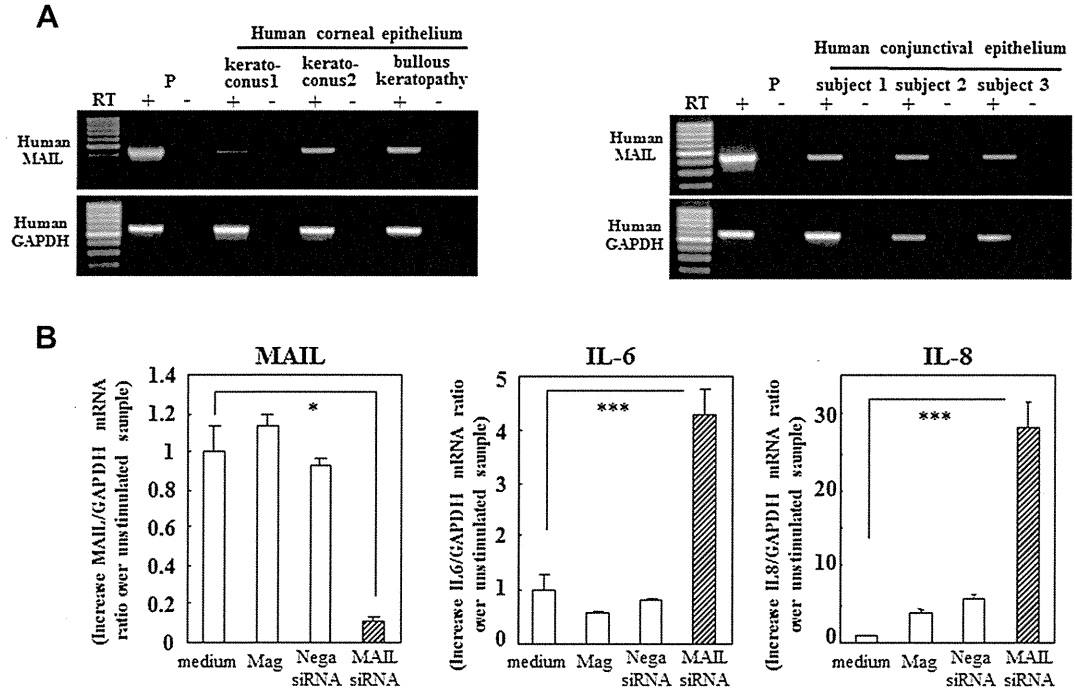

Fig. 17. Expression of MAIL (similar to mouse IκBζ)-mRNA on the human ocular surface. A. RT-PCR detected MAIL-specific mRNA in the human corneal and conjunctival epithelium. Human peripheral monocytes stimulated with 100 ng/ml LPS were the positive control. (P: adherent mononuclear cells stimulated with 100 ng/ml LPS). B. Knock down of MAIL enhanced the expression of IL-6- and IL-8-specific mRNA. For the transfection of small interfering RNA (siRNA), 1 μg/ml of the control- or targeting siRNA was transfected into primary human corneal epithelial cells using PolymagII (OZ BIOSCIENCES) according to the manufacturer's recommendations. Mag: only PolymagII, Nega siRNA: control siRNA. (*, $p < 0.05$; ***, $p < 0.005$). Reprinted with permission from Ueta et al. (Ueta et al., 2005b; Ueta and Kinoshita, 2010a).

Many patients encountered by ophthalmologists present in the chronic stage of SJS/TEN; dermatologists tend to see patients with SJS/TEN in the acute stage. The differential diagnosis of SJS or TEN may be difficult in the chronic stage of SJS/TEN because at that point the vesiculobullous skin lesions present in the acute stage have healed. Thus, ophthalmologists tend to diagnose both SJS and TEN as SJS in the broad sense. Our 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 (Sotozono et al., 2007, 2009a; Ueta and Kinoshita, 2010a; Ueta et al., 2007c, 2008b,, 2007e, 2008d; c; Ueta et al., 2007d, 2010c, 2007e, 2008d).

The pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established. The extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007c, 2008b,, 2012b, 2008d; c; Ueta et al., 2007d, 2010c, 2007e, 2012b, 2008d).

6.2. HLA analysis of SJS with ocular surface complications

In 1982, ophthalmologists first reported that the HLA-Bw44 antigen, a subgroup of HLA-B12, was significantly increased in Caucasian patients with SJS with ocular involvement compared with a control Caucasian population. In that study, the onset of SJS with ocular involvement was associated with putative viral syndromes or the administration of drugs (Mondino et al., 1982). Dermatologists also found that the frequency of the HLA-B12 antigen was significantly increased in French SJS/TEN patients whose disorder was clearly drug-induced compared with a French control population; the main causative agents were non-steroidal anti-inflammatory drugs (NSAIDs) (Roujeau et al., 1986).

We examined HLA-class I (HLA-A, -B, -C) antigens in Japanese SJS patients with severe ocular surface complications (Ueta et al., 2007e, 2008d) and found that the carrier frequency of the HLA-A*0206 antigen was significantly higher in 110 SJS patients compared to 220 Japanese controls (carrier frequency: 45.5% vs 13.6%, $p = 0.0000000002$, odds ratio (OR) = 5.3, gene frequency: 23.6% vs 6.8%, $p = 0.0000000007$, OR = 4.2). However, HLA-A*0206, strongly associated with SJS/TEN with ocular complications in Japanese individuals, is absent in Caucasians (Ueta et al., 2007e, 2008d).

On the other hand, in our study, as in earlier reports (Mondino et al., 1982; Yetiv et al., 1980), the onset of SJS with severe ocular surface complications was associated with putative viral syndromes and/or the administration of drugs (mainly NSAIDs) (Ueta et al., 2007c, 2008b, 2007d, 2010c, 2007e). We found no association with HLA-B12 in Japanese SJS patients (Kaniwa et al., 2008; Ueta et al., 2007e, 2008d) although this antigen was significantly increased in Caucasian SJS patients (Mondino et al., 1982; Roujeau et al., 1986), probably because in Caucasians the HLA-B12 antigen is primarily coded by HLA-B*4402 whereas in Japanese it is almost exclusively coded by HLA-B*4403 (Tokunaga et al., 1997).

Thus, our findings suggest strong ethnic differences in the association of SJS/TEN and HLA (Ueta et al., 2007e, 2008d). Specific combinations of genes and certain environmental factors may be required for the manifestation of this rare phenotype because SJS/TEN is rare and it probably has a complex genetic inheritance background (Ueta et al., 2007e, 2008d).

In Han Chinese (Chung et al., 2004) but not in Caucasian patients (Lonjou et al., 2008, 2006) there was a strong carbamazepine-specific association between HLA-B*1502 and carbamazepine-induced SJS/TEN. Because the allele frequency of HLA-B*1502 is very low in the Japanese, the carbamazepine-specific association between HLA and carbamazepine-induced SJS may be specific for certain ethnic groups (Kaniwa et al., 2008; Ueta et al., 2008d).

Although an allopurinol-specific association between HLA-B*5801 and allopurinol-induced severe cutaneous adverse reactions

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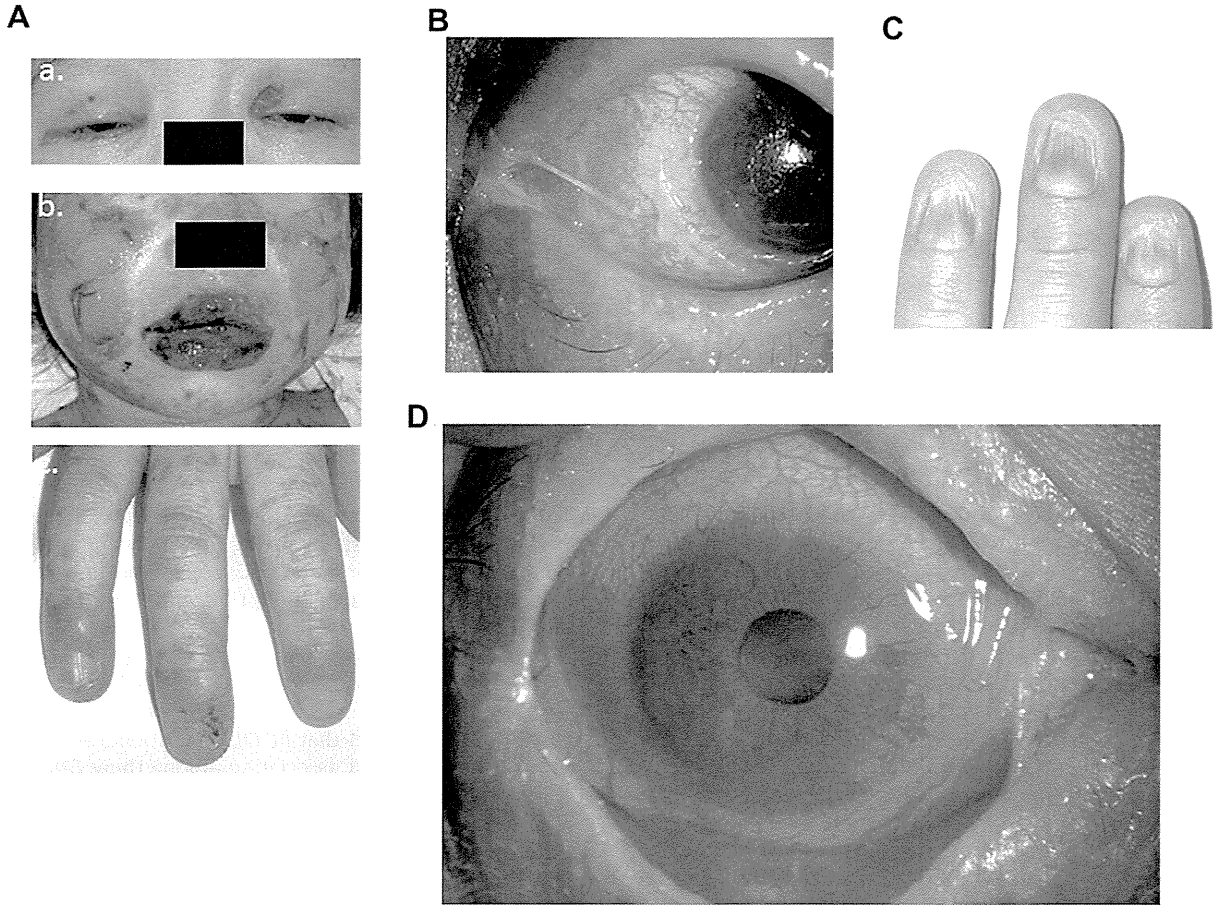

Fig. 18. Stevens-Johnson syndrome (SJS) with severe ocular surface complications. A. Typical features of SJS/TEN in the acute stage. a. Ocular surface inflammation with conjunctivitis and eyelids swelling. b. The face manifests swollen and crusted lips, blisters, and erosion of the skin. c. Paronychia. Reprinted with permission from Ueta et al. (Ueta and Kinoshita, 2010a). B. Ocular surface inflammation of SJS: severe conjunctivitis, pseudomembrane, epithelial defect, etc. C. Transformed fingernails in the chronic stage. D. Ocular surface complications in the chronic stage; conjunctival invasion into the cornea, symblepharon, trichiasis, and dry eye.

may be a universal phenomenon in all ethnic groups (Hung et al., 2005) allopurinol-induced severe adverse cutaneous reactions may not elicit serious sequelae on the ocular surface. In fact, few SJS patients with severe ocular surface complications manifested allopurinol-related SJS/TEN (Kaniwa et al., 2008).

Drugs are probably the most widely accepted etiologic factors in SJS/TEN (Levi et al., 2009; Mockenhaupt et al., 2008; Roujeau et al., 1995; Wolf et al., 2005). It is worth noting that our SJS/TEN patients with severe ocular surface complications often presented with prodromata including nonspecific fever, coryza, and sore throat that closely mimic upper respiratory tract infections commonly treated with antibiotics and NSAIDs (Ueta and Kinoshita, 2010a; Ueta et al., 2007d, 2010c). More than 80% of our SJS patients developed SJS after receiving treatment for the common cold with antibiotics, cold remedies, and/or NSAIDs; only about 5% progressed to SJS after drug treatment delivered to prevent the occurrence of convulsions (Ueta et al., 2010c).

6.3. Gene expression analysis of SJS with ocular surface complications

We have proposed the possibility of an association between a disordered innate immune response and SJS with severe ocular surface complication. Our hypothesis was based on the observation of an association between the onset of the SJS and microbial infections, because many SJS patients with severe ocular surface complications exhibited prodromata, including non-specific fever, coryza, and sore throat, ailments that closely mimic upper respiratory tract infections of viral or mycoplasma origin, which are commonly treated with antibiotics and NSAIDs (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007d, 2010c, 2012b). In addition, the SJS patients presented with opportunistic infection of the ocular surface by bacteria, especially methicillin-resistant *S. aureus* and *Staphylococcus epidermis* (MRSA and MRSE); the detection rate of MRSA and MRSE was higher on the ocular surface of SJS/TEN patients compared to individuals with other devastating ocular surface disorders (Sotozono et al., 2002). We posit that in SJS/TEN patients, opportunistic infections of the ocular surface by bacteria are ascribable to abnormalities in their innate immunity. Moreover, SJS/TEN patients presented with persistent inflammation of the ocular surfaces harboring commensal bacteria.

Under the hypothesis of a disordered innate immune response in SJS with ocular complications, we performed gene expression analysis of monocytes, which are essential in innate immunity. We found differences in IL4R gene expression; it was down-regulated in SJS/TEN patients upon LPS stimulation and slightly up-regulated in the controls (Fig. 19A) (Ueta, 2008). We also found that in human ocular surface (corneal and conjunctival) epithelial cells IL4R-specific mRNA was down-regulated upon stimulation with PolyI:C which mimics viral components (data not shown). This observation suggests that IL4R is linked with innate immunity (Ueta, 2008). We also found that after 1-hr culture without LPS, the expression of IκBζ- and IL-1α-specific mRNA was lower in monocytes from SJS/TEN patients than normal controls (Fig. 19B, C) (Ueta,

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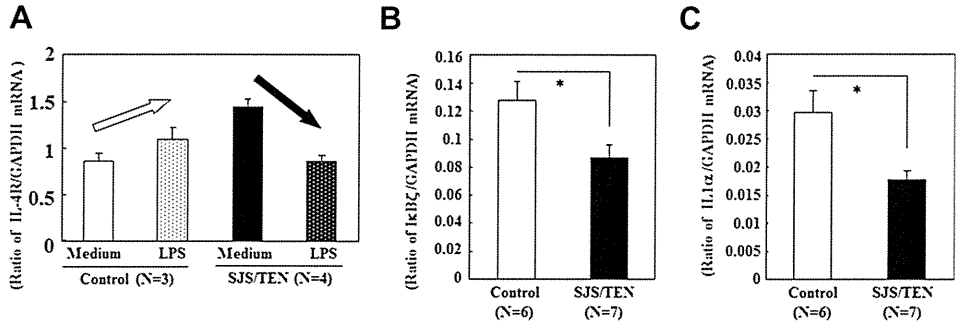

Fig. 19. Gene expression analysis of monocytes ($CD14^+$ cells) from SJS with ocular complications. A: Difference in IL4R gene expression between SJS patients and normal volunteers. $CD14^+$ cells from peripheral blood were subjected to gene expression analysis. The cells were cultured for 1 h with or without LPS. B, C. Low expression of IκBζ and IL-1α by isolated monocytes from SJS patients after 1-hr culture. Quantitative RT-PCR assay confirmed that IκBζ (B) and IL-1α (C) gene expression was significantly lower in cultured monocytes from 7 SJS/TEN patients than the 6 controls. Data show the mean ± SEM. (*, $p < 0.05$; ***, $p < 0.005$); evaluation was with Student's *t*-test using the Excel program. Reprinted with permission from Ueta et al. (Ueta, 2008).

2008). This suggests that the reduced expression of IκBζ and IL-1α genes may play an important role in the pathophysiology of SJS/TEN.

Possibly to prevent excessive inflammation in the presence of bacterial components, IκBζ induced by diverse pathogen-associated molecular patterns regulates NF-κB activity (Yamazaki et al., 2001). Elsewhere we documented that IκBζ gene-disrupted mice manifested ocular surface inflammation (Ueta et al., 2008a, 2005b) and that IκBζ in the ocular surface epithelium can suppress the production of pro-inflammatory cytokines such as IL-6 and IL-8 (Ueta and Kinoshita, 2010a). This suggests that the ocular surface epithelium suppresses inflammation via the expression of IκBζ (Ueta et al., 2008a, 2005b; Ueta and Kinoshita, 2010a).

We also reported that the TLR3 ligand, elicited the elevated expression of human IκBζ-specific mRNA in ocular surface epithelial cells (Ueta et al., 2005a). Because TLRs could induce the expression of IκBζ (Ueta et al., 2005a), the ocular surface inflammation seen in SJS/TEN patients may be related to innate pathogen-associated molecular pattern-amplified immune responses to microbes.

IL-1α was significantly lower and sIL-2R significantly higher in the blister fluid of TEN- than burn patients (Correia et al., 2002). We also detected a significant difference between SJS/TEN patients and the controls with respect to the expression of IL-1α by $CD14^+$ monocytes (Ueta, 2008; Ueta and Kinoshita, 2010a).

6.4. Single nucleotide polymorphism (SNP) analysis of SJS with severe ocular surface complications

6.4.1. The candidate gene approach

While the administration of some drugs may result in the development of SJS/TEN, not all patients taking these drugs develop SJS/TEN. As the incidence of SJS/TEN is very low, we suspected a genetic predisposition (Ueta, 2008; Ueta and Kinoshita, 2010a). We therefore performed SNP association analysis using candidate genes associated with innate immunity (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007d), allergy (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007c, 2008b), or apoptosis (Ueta et al., 2008c).

We first examined candidate genes associated with innate immunity. We investigated the IκBζ gene, which yielded different findings for SJS/TEN patients and controls in our gene expression analysis, and whose disruption results in ocular surface and skin inflammation. Another was the IL1α gene which, based on gene expression analysis, is also different in SJS/TEN patients and the controls. Other candidate genes were the TLR2 gene which is closely related to *S. aureus* and *S. epidermidis*, including MRSA and MRSE, and the TLR3 gene which is the gene most highly expressed on ocular surface epithelium among 1–10 TLRs and which responds to the virus dsRNA-mimic polyI:C to generate pro-inflammatory cytokines and IFN-β (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007d).

To investigate IκBζ we analyzed 7 polymorphisms (rs.2305991, rs.622122, rs.14134, rs.3217713, rs.595788, rs.677011, rs.3821727) in the Japanese Single Nucleotide Polymorphisms (JSNP) database (Ueta, 2008). We found that in 110 SJS patients and 220 controls there was no significant association among these SNPs. Regarding IL1α, we analyzed 5 SNPs (rs.1609682, rs.1894399, rs.2071373, rs.2071375, rs.2071376) reported in JSNP. Again, we found no significant association among these SNPs (Ueta, 2008). For TLR2 we analyzed 3 SNPs (rs.3840100, rs.3840099, rs.3840097) in JSNP and found no significant association among these SNPs (Ueta, 2008). However, our analysis of 7 SNPs for TLR3 (rs.3775290, rs.3775291, rs.3775292, rs.3775293, rs.3775294, rs.3775295, rs.3775296) (Ueta et al., 2007d) revealed that in 110 SJS patients and 220 controls, SNP rs.3775296 showed a significant association under a recessive model (rs.3775296T/T vs T/G + G/G, p value = 0.00004, OR = 4.3) and a weak association with allele frequency (T vs G, p value = 0.006, OR = 1.6) and SNP rs.3775290 also showed a significant association under a recessive model (rs.3775290 A/A vs A/G + G/G, p value = 0.007, OR = 2.3) (Fig. 20). Thus, our findings suggested that polymorphisms in the TLR3 gene are associated with SJS with severe ocular surface complications in the Japanese population (Ueta et al., 2007d).

We have reported that human ocular surface epithelial cells strongly expressed TLR3 and that its ligand, polyI:C could induce various molecules such as pro-inflammatory cytokines and anti-viral- and allergy-related-molecules (Ueta et al., 2005a, 2010b; Ueta and Kinoshita, 2010a, 2010b). Elsewhere we offered the hypothesis 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 mucosa, ocular surface, and skin (Ueta, 2008; Ueta and Kinoshita, 2010a; Ueta et al., 2007d, 2012b).

Next we examined candidate genes associated with allergy. Our gene expression analysis had shown that with respect to the IL4R gene there are differences between SJS patients and the controls (Ueta, 2008). This gene is essential for both IL-4 and IL-13 signaling because it is a component of IL-4 and IL-13 receptors. We analyzed Gln551Arg (rs.1801275), Ile50Val (rs.1805010), and Ser478Pro (rs.1805015) polymorphisms of IL4R as they are associated with allergic diseases such as asthma (Ueta et al., 2007c, 2008b). We found no significant association between Ile50Val (rs.1805010), and Ser478Pro (rs.1805015) (Ueta et al., 2007c, 2008b). On the other hand, Gln551Arg was significantly associated with allele frequency

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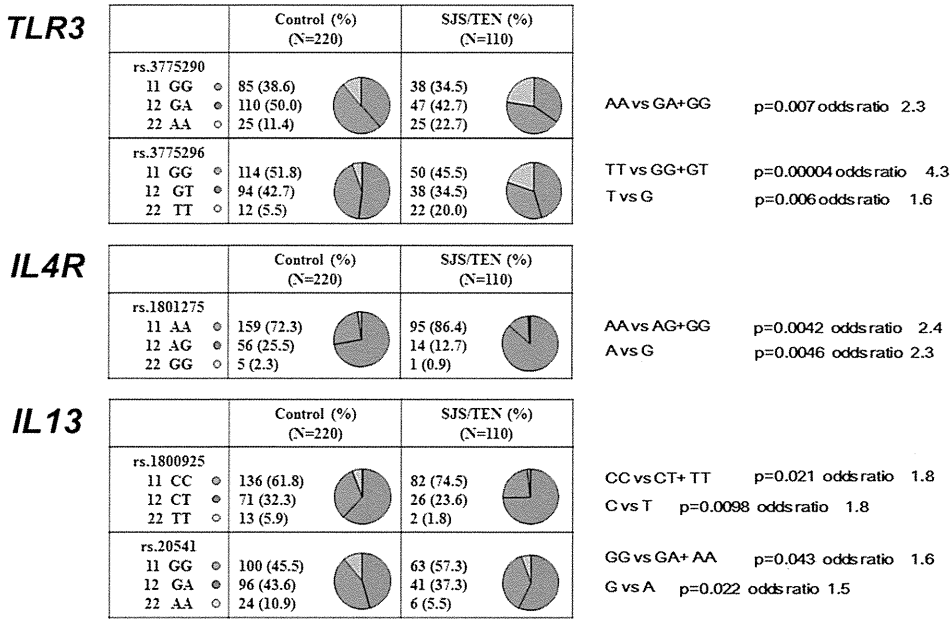

Fig. 20. Association between TLR3, IL4R, and IL13 SNPs and SJS with severe ocular surface complications. Using the candidate gene approach we identified SNPs of TLR3, IL4R and IL13 genes that were associated with SJS with severe ocular surface complications.

(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 SJS patients and the 220 controls (Fig. 20)(Ueta et al., 2007c, 2008b).

We also investigated IL13 and IL4, ligands of IL4R. With respect to the IL13 gene we analyzed polymorphisms of the promoter −1111C/T SNP (rs.1800925) and the Gln110Arg SNP (rs.20541); they are associated with allergic diseases such as asthma. There was a significant association of the promoter −1111C/T SNP with allele frequency (C vs T, p value = 0.0098, OR = 1.8) in all 110 SJS patients and the 220 controls; the Gln110Arg SNP exhibited a significant association with allele frequency (G vs A, p value = 0.022, OR = 1.5)(Fig. 20). We detected a significant increase in Arg110 in our SJS/TEN patients (Ueta et al., 2008b), although Gln110 was significantly increased in patients with asthma (Heinzmann et al., 2000).

With respect to the IL4 gene we analyzed polymorphisms of the promoter −590C/T (rs.2243250) related to higher IgE levels. We found no significant association between the SJS patients and the controls (Ueta et al., 2008b).

Lastly we examined FasL genes, the candidate genes associated with apoptosis; they have been reported to be increased in the serum of SJS/TEN patients in the acute stage (Abe et al., 2003). We examined 4 SNPs (rs.929087, rs.2639614, rs.2859247, rs.3830150) and found that rs.3830150 A/G (intron) showed a weak association with the dominant model (A/G + G/G vs A/A, p value = 0.015, OR = 1.8) in 110 SJS patients and 220 controls (Ueta et al., 2008c).

In summary, we found that TLR3 rs.3775296 SNP, IL4R SNP rs.1801275 (Gln551Arg), and IL13 rs.20541 (Arg110Gln) were significantly associated with SJS/TEN with ocular surface complications (Ueta, 2008; Ueta and Kinoshita, 2010a).

6.4.2. Genome-wide association study (GWAS)

To elucidate the pathophysiology of SJS with severe ocular surface complications in more detail 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 detected 3 SNPs (rs1325975: chr6, rs17131450: chr1, rs11238074: chr11) that were significantly associated with SJS with severe ocular surface complications. Because 2 of the SNPs (rs1325975 and rs11238074) were from the "gene desert" region, we focused on a SNP (rs17131450) that mapped close to the *PTGER3* gene, which is the gene of *EP3* protein of human, located in the 1p31 region of the human genome (Ueta et al., 2010c).

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* gene through the two major linkage disequilibrium (LD) blocks of the HapMap Japanese (JPT) plus the Han Chinese (CHB) population. The rs17131450 SNP showing a significant association with SJS in the GWAS also showed a significant association ($p < 0.01$) in our fine-mapping analysis. We also identified 5 other significantly associated ($p < 0.01$) SNPs in *PTGER3* gene (rs5702, rs1325949, rs7543182, rs7555874, and rs4147114) (Ueta et al., 2010c). One of the 6 SNPs in *PTGER3* gene (rs5702) was in an exon as a silent SNP (sSNP), four (rs1325949, rs7543182, rs7555874, rs4147114) were in introns (iSNPs), and the remaining SNP (rs17131450) was a genome SNP (Ueta et al., 2010c). Lastly we assessed the association of the 6 SNPs by direct sequencing (Ueta et al., 2010c). A summary of our case–control analysis based on sequence data from 110 SJS patients and 220 control subjects is shown in Fig. 21. Based on our GWAS and direct sequencing analysis we identified 6 SNPs associated with SJS/TEN, 5 of these were located within the *PTGER3* gene (Ueta et al., 2010c).

Because EP3, which is the protein of PTGER3 gene, is constitutively expressed in mouse conjunctival epithelial cells (Ueta et al., 2009a) we examined its expression in normal human conjunctival epithelial cells. RT-PCR assay showed that normal human conjunctival epithelial cells expressed *PTGER3* mRNA and immunohistochemistry disclosed the presence of EP3 protein (Ueta et al., 2010c, 2011d). When we looked for the expression of EP3 in the conjunctival epithelium of SJS/TEN patients with severe ocular surface complications we did not find EP3 protein. On the other hand, the protein was present in the control conjunctival epithelium from patients with conjunctivochalasis or pterygium (Ueta et al., 2010c, 2011d).

In support of the genetic association of *PTGER3* gene polymorphisms and SJS with severe ocular surface complications, we found that compared to the controls, the expression of EP3 protein

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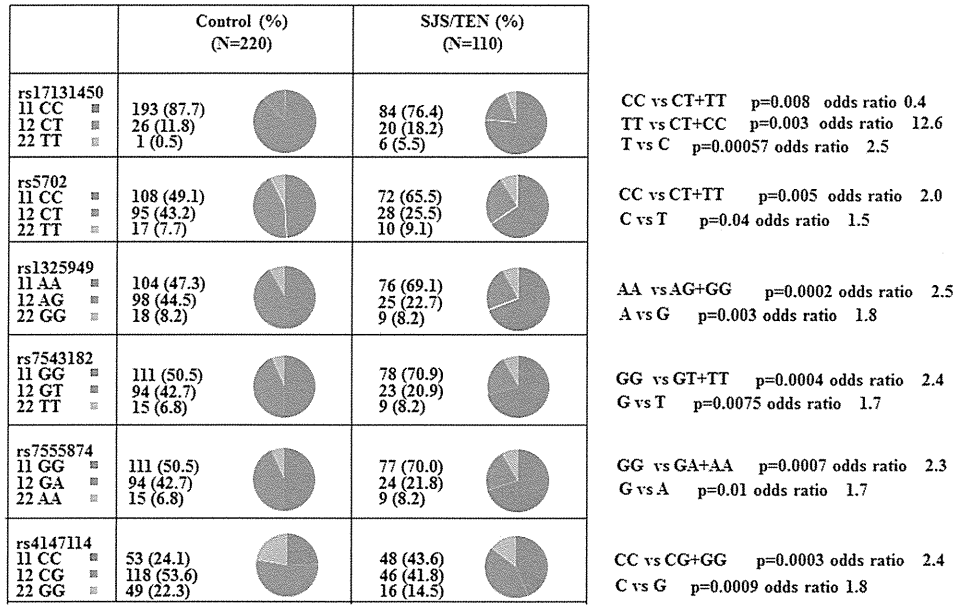

Fig. 21. Association between PTGER3 SNPs and SJS with severe ocular surface complications. Using the genome wide association study (GWAS) we found that 6 SNPs of the *PTGER3* gene were associated with SJS with severe ocular surface complications.

was greatly reduced in the conjunctival epithelium of these patients. This suggests that EP3 contributes functionally to the pathogenesis of SJS/TEN (Ueta et al., 2010c, 2011d).

Based on the finding that more than 75% of our SJS patients had used cold medications, possibly including NSAIDs, before the onset of their disease we posited that the observed *PTGER3* polymorphisms are associated with a NSAID-related susceptibility to SJS with severe ocular surface complications (Ueta et al., 2010c). Drugs are probably the most widely accepted etiologic factor for SJS (Roujeau et al., 1995); in fact, many patients who develop SJS with severe ocular involvement do so after taking remedies for the common cold or NSAIDs, drugs that inhibit the production of the EP3 ligand, PGE_2. This observation supports the hypothesis that EP3 is involved in the development of SJS with severe ocular surface involvement.

6.4.3. *Interaction between the TLR3 and the EP3*

We reported that polymorphisms in *PTGER3*, the gene of EP3, were significantly associated with SJS with severe ocular surface complications (Ueta et al., 2010c), that PGE_2 is a ligand for EP3 in

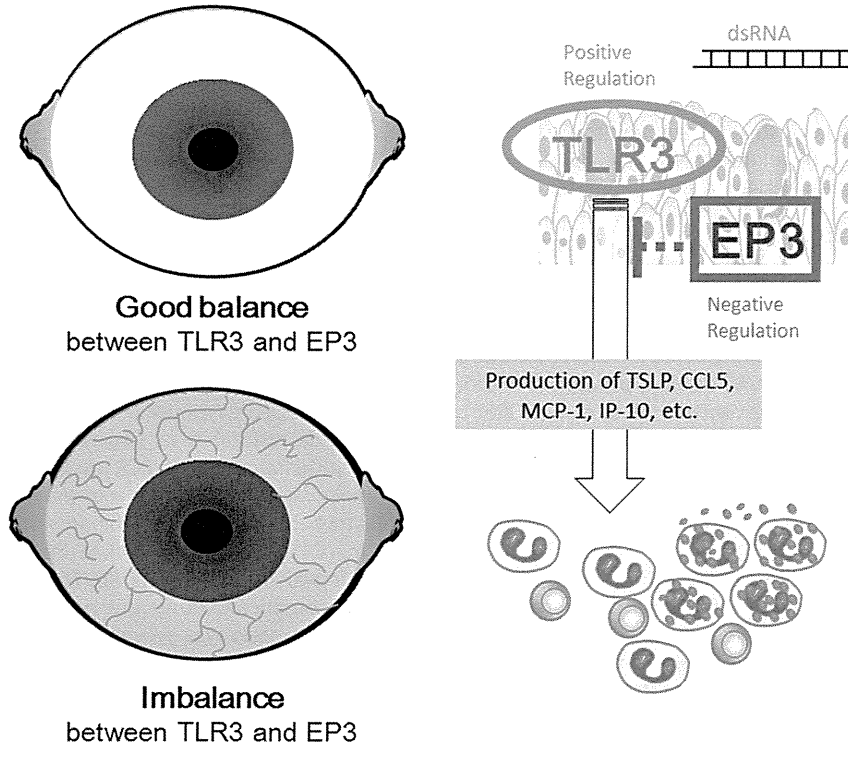

Fig. 22. Lack of balance between TLR3 and EP3 might trigger ocular surface inflammation.

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the conjunctival epithelium, and that the PGE_2-EP3 pathway down-regulates the progression of murine EAC (Ueta et al., 2009a). We also documented that TLR3 polymorphisms are associated with SJS (Ueta et al., 2007d), that the human ocular surface epithelium strongly expresses TLR3, and that cytokine production is up-regulated by polyI:C, a TLR3 ligand (Ueta, 2008; Ueta et al., 2005a; Ueta and Kinoshita, 2010a). 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 agonist significantly suppressed the production and mRNA expression of CCL5, CXCL10, CXCL11, IL-6, TSLP, and MCP-1 in polyI:C-stimulated primary human conjunctival epithelial cells, suggesting that cytokine production by conjunctival epithelial cells in response to polyI:C stimulation can be suppressed through the activation of EP3 (Ueta et al., 2011b, c; Ueta et al., 2012a).

In the past decade, SNPs were widely used as genetic markers for identifying human disease-susceptibility genes. It is now apparent that gene–gene interactions should be considered in addition to major single-locus effects (Cordell, 2009). In particular, non-additive (epistatic) models for some complex diseases fit with actual observations, suggesting interactions involving multiple loci (Ritchie et al., 2001). We performed a statistical search for interactions between all possible pairs of loci by applying high-dimensional variable selection methods to the comprehensive dataset obtained from our previous studies that involved a total of 14 immune-related genes including *PTGER3 and TLR3*. We found a variable with susceptible effects on SJS; these effects were involved in locus-pairs of *PTGER3-TLR3*. The *PTGER3* rs.4147114G/C SNP and the *TLR3* rs.3775296T/T SNP exhibited a higher odds ratio (OR: 25.3, $p = 0.0000527$) than only *TLR3* rs.3775296T/T SNP (OR: 5.35, $p = 0.00025$) or only *PTGER3* rs.4147114G/C SNP (OR: 2.66, $p = 0.0023$) (Ueta et al., 2012b).

Next we focused on the epistatic interaction between *PTGER3* and *TLR3* and analyzed an additional 32 SNPs of *PTGER3* and 10 SNPs of *TLR3* (a total of 38 SNPs of *PTGER3* and 17 SNPs of *TLR3*). We found that besides the previously reported 6 *PTGER3*- and 2 *TLR3*-SNPs, 14 additional *PTGER3* SNPs and 5 additional *TLR3* SNPs were associated with SJS with severe ocular surface complications (Ueta et al., 2012b).

Elsewhere we showed that conjunctival eosinophilic infiltration in EAC was significantly more marked in EP3-KO mice (Ueta et al., 2009a) and significantly less marked in TLR3-KO mice than in wild-type mice (Ueta et al., 2009c). We also reported that 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 (Ueta et al., 2012b). These findings suggest that in EAC, EP3 negatively regulates the eosinophilic infiltration induced by TLR3 (Ueta et al., 2012b).

Thus, we provide evidence that there are functional interactions between TLR3 and EP3 that exert susceptibility effects with respect to SJS with severe ocular surface complications and that the

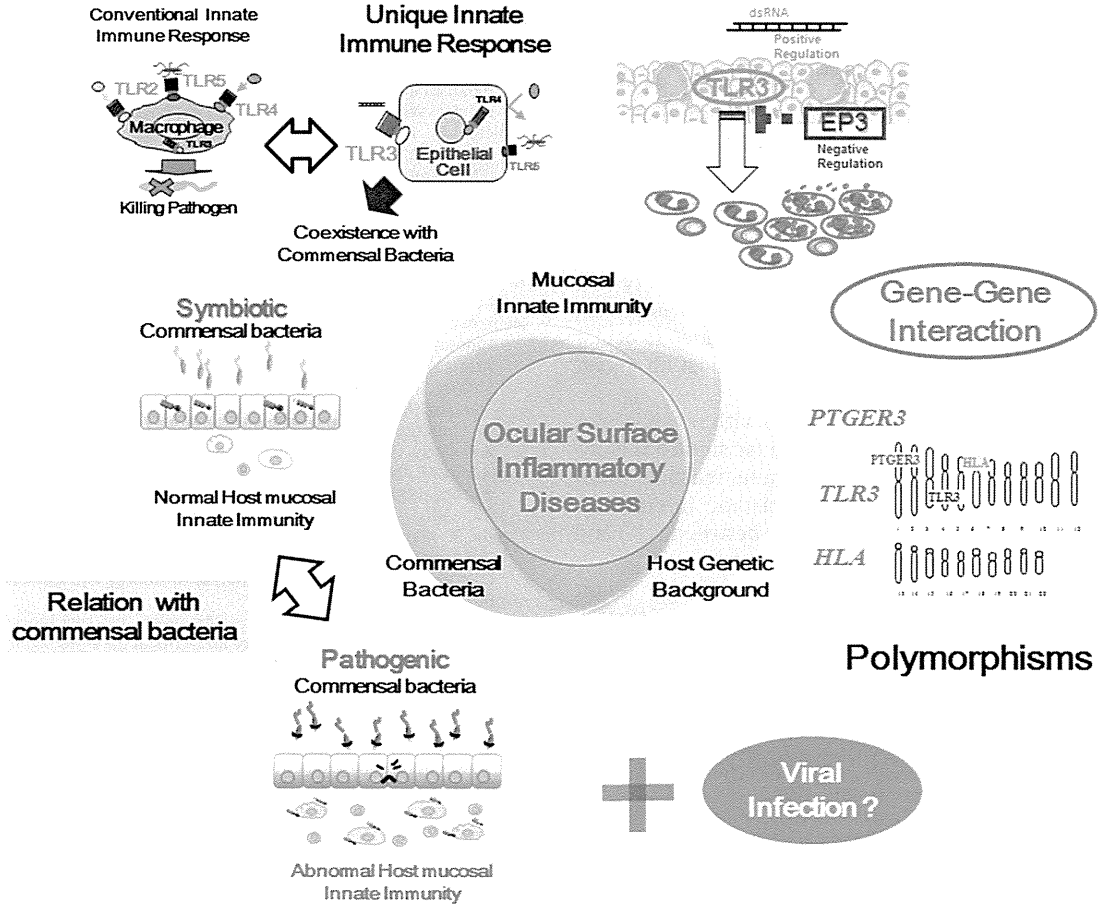

Fig. 23. The presumed pathophysiological mechanism of the ocular surface inflammatory diseases. Ocular surface inflammatory diseases are involved with mucosal innate immunity, commensal bacteria, and host genetic background. Unique innate immune response of epithelial cells contributes to coexistence with commensal bacteria. The pathogenicity of commensal bacteria is influenced by the abnormal condition of host mucosal innate immunity. Host genetic background such as polymorphisms is involved with host mucosal innate immunity. Gene–gene interactions also contribute to pathobiological mechanisms of human ocular surface inflammatory diseases.

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interactions are epistatic (Ueta et al., 2012b). Based on the findings discussed here we strongly suspect that the lack of balance between TLR3 and EP3 can trigger ocular surface inflammation (Fig. 22).

7. Conclusions and future directions

In this review we raise the possibility that some ocular surface inflammatory diseases are pathogenetically related with a disordered innate immune response.

Although the ocular surface epithelium is in constant contact with bacteria and bacterial products, the healthy ocular surface is not in an inflammatory state. The balance between the mucosal immunity of the ocular surface and the pathogenicity of bacteria is very important. We suspect that when the host mucosal immunity is normal, commensal bacteria are in a symbiotic relationship with their host, however, if the host mucosal immunity is abnormal, commensal bacteria may become pathogenic. Some ocular surface inflammatory diseases such as catarrhal ulcers and phlyctenular keratitis are considered to be hypersensitivity to bacteria.

We also showed that although immune-competent cells such as macrophages could recognize various microbial components through various TLRs, induce inflammation and then exclude the microbes, ocular surface epithelial cells can selectively respond to microbial components and induce limited inflammation. We suspect that the difference between ocular surface epithelial cells and macrophages lies in their dissimilarity with respect to their coexistence with commensal bacteria. The unique innate immune response machinery of the ocular surface epithelium may explain the permissive coexistence with commensal bacteria. We also document that human ocular surface epithelial cells can be induced upon stimulation with polyI:C, a ligand of TLR3, RIG-I and MDA-5, to express many transcripts including not only anti-viral innate immune response-related- but also allergy-related-genes.

We provided evidence that allergic eosinophilic infiltration of the conjunctiva can be regulated by conjunctival epithelial cells through EP3 and TLR3.

Our findings indicate that disordered innate immunity can induce ocular surface inflammation because mice in which IκBζ was knocked out, expressly exhibited severe, spontaneous ocular surface inflammation with the eventual loss of almost all goblet cells.

Lastly we suggest that the pathogenesis of SJS with severe ocular surface complications, a devastating severe ocular surface inflammatory disease, is associated with innate immune reaction abnomalies, especially those related with the epistatic interactions between TLR3 and EP3. Thus, the lack of balance between TLR3 and EP3 might trigger ocular surface inflammation.

Focusing on the innate immunity of the ocular surface might help to elucidate the pathogenesis of various ocular surface diseases (Fig. 23).

References

Abe, R., Shimizu, T., Shibaki, A., Nakamura, H., Watanabe, H., Shimizu, H., 2003. Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. American Journal of Pathology 162, 1515–1520.

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A., 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732–738.

Auquier-Dunant, A., Mockenhaupt, M., Naldi, L., Correia, O., Schroder, W., Roujeau, J.C., 2002. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. Archives of Dermatology 138, 1019–1024.

Baum, J.L., 1978. Current concepts in ophthalmology. Ocular infections. The New England Journal of Medicine 299, 28–31.

Beauchamp, G.R., Gillette, T.E., Friendly, D.S., 1981. Phlyctenular keratoconjunctivitis. Journal of Pediatric Ophthalmology and Strabismus 18, 22–28.

Bouma, G., Strober, W., 2003. The immunological and genetic basis of inflammatory bowel disease. Nature Reviews Immunology 3, 521–533.

Broide, D.H., 2007. The pathophysiology of allergic rhinoconjunctivitis. Allergy and Asthma Proceedings: the Official Journal of Regional and State Allergy Societies 28, 398–403.

Bullens, D.M., Decraene, A., Dilissen, E., Meyts, I., De Boeck, K., Dupont, L.J., Ceuppens, J.L., 2008. Type III IFN-lambda mRNA expression in sputum of adult and school-aged asthmatics. Clinical and Experimental Allergy 38, 1459–1467.

Cavassani, K.A., Ishii, M., Wen, H., Schaller, M.A., Lincoln, P.M., Lukacs, N.W., Hogaboam, C.M., Kunkel, S.L., 2008. TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. The Journal of Experimental Medicine 205, 2609–2621.

Cho, J.H., 2008. The genetics and immunopathogenesis of inflammatory bowel disease. Nature Reviews Immunology 8, 458–466.

Chung, W.H., Hung, S.I., Hong, H.S., Hsih, M.S., Yang, L.C., Ho, H.C., Wu, J.Y., Chen, Y.T., 2004. Medical genetics: a marker for Stevens-Johnson syndrome. Nature 428, 486.

Cordell, H.J., 2009. Detecting gene-gene interactions that underlie human diseases. Nature Reviews Genetics 10, 392–404.

Correia, O., Delgado, L., Roujeau, J.C., Le Cleach, L., Fleming-Torrinha, J.A., 2002. Soluble interleukin 2 receptor and interleukin 1alpha in toxic epidermal necrolysis: a comparative analysis of serum and blister fluid samples. Archives of Dermatology 138, 29–32.

Doyle, A., Beigi, B., Early, A., Blake, A., Eustace, P., Hone, R., 1995. Adherence of bacteria to intraocular lenses: a prospective study. The British Journal of Ophthalmology 79, 347–349.

Farber, J.M., 1997. Mig and IP-10: CXC chemokines that target lymphocytes. Journal of Leukocyte Biology 61, 246–257.

Ficker, L., Seal, D., Wright, P., 1989. Staphylococcal infection and the limbus: study of the cell-mediated immune response. Eye (Lond) 3, 190–193.

Forman, R., Koren, G., Shear, N.H., 2002. Erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. Drug Safety 25, 965–972.

Fukuda, K., Kumagai, N., Fujitsu, Y., Nishida, T., 2006. Fibroblasts as local immune modulators in ocular allergic disease. Allergology International 55, 121–129.

Fukushima, A., 2007. Roles of T-cells in the development of allergic conjunctival diseases. Cornea 26, S36–S40.

Graziano, F.M., Stahl, J.L., Cook, E.B., Barney, N.P., 2001. Conjunctival mast cells in ocular allergic disease. Allergy Asthma Proc 22, 121–126.

Gros, E., Bussmann, C., Bieber, T., Forster, I., Novak, N., 2009. Expression of chemokines and chemokine receptors in lesional and nonlesional upper skin of patients with atopic dermatitis. The Journal of Allergy and Clinical Immunology.

Hallen, L.C., Burki, Y., Ebeling, M., Broger, C., Siegrist, F., Oroszlan-Szovik, K., Bohrmann, B., Certa, U., Foser, S., 2007. Antiproliferative activity of the human IFN-alpha-inducible protein IFI44. J Interferon Cytokine Res 27, 675–680.

Hara, J., Yasuda, F., Higashitsutsumi, M., 1997. Preoperative disinfection of the conjunctival sac in cataract surgery. Ophthalmologica. Journal International D'ophtalmologie. International Journal of Ophthalmology. Zeitschrift fur Augenheilkunde 211 (Suppl. 1), 62–67.

Haynes, R.J., Tighe, P.J., Dua, H.S., 1999. Antimicrobial defensin peptides of the human ocular surface. The British Journal of Ophthalmology 83, 737–741.

Heinzmann, A., Mao, X.Q., Akaiwa, M., Kreomer, R.T., Gao, P.S., Ohshima, K., Umeshita, R., Abe, Y., Braun, S., Yamashita, T., Roberts, M.H., Sugimoto, R., Arima, K., Arinobu, Y., Yu, B., Kruse, S., Enomoto, T., Dake, Y., Kawai, M., Shimazu, S., Sasaki, S., Adra, C.N., Kitaichi, M., Inoue, H., Yamauchi, K., Tomichi, N., Kurimoto, F., Hamasaki, N., Hopkin, J.M., Izuhara, K., Shirakawa, T., Deichmann, K.A., 2000. Genetic variants of IL-13 signalling and human asthma and atopy. Human Molecular Genetics 9, 549–559.

Hingorani, M., Calder, V.L., Buckley, R.J., Lightman, S.L., 1998. The role of conjunctival epithelial cells in chronic ocular allergic disease. Experimental Eye Research 67, 491–500.

Honda, T., Matsuoka, T., Ueta, M., Kabashima, K., Miyachi, Y., Narumiya, S., 2009. Prostaglandin E(2)-EP(3) signaling suppresses skin inflammation in murine contact hypersensitivity. The Journal of Allergy and Clinical Immunology 124, 809–818. e802.

Horisberger, M.A., 1995. Interferons, Mx genes, and resistance to influenza virus. American Journal of Respiratory and Critical Care Medicine 152, S67–S71.

Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., Endres, S., Hartmann, G., 2002. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. The Journal of Immunology 168, 4531–4537.

Hozono, Y., Ueta, M., Hamuro, J., Kojima, K., Kawasaki, S., Yamazaki, K., Kinoshita, S., 2006. Human corneal epithelial cells respond to ocular-pathogenic, but not to nonpathogenic-flagellin. Biochemical and Biophysical Research Communications 347, 238–247.

Hung, S.I., Chung, W.H., Liou, L.B., Chu, C.C., Lin, M., Huang, H.P., Lin, Y.L., Lan, J.L., Yang, L.C., Hong, H.S., Chen, M.J., Lai, P.C., Wu, M.S., Chu, C.Y., Wang, K.H., Chen, C.H., Fann, C.S., Wu, J.Y., Chen, Y.T., 2005. HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proceedings of the National Academy of Sciences of the United States of America 102, 4134–4139.

Jin, H.K., Yoshimatsu, K., Takada, A., Ogino, M., Asano, A., Arikawa, J., Watanabe, T., 2001. Mouse Mx2 protein inhibits hantavirus but not influenza virus replication. Archives of Virology 146, 41–49.

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Johnson, A.C., Heinzel, F.P., Diaconu, E., Sun, Y., Hise, A.G., Golenbock, D., Lass, J.H., Pearlman, E., 2005. Activation of toll-like receptor (TLR)2, TLR4, and TLR9 in the mammalian cornea induces MyD88-dependent corneal inflammation. Investigative Ophthalmology & Visual Science 46, 589–595.

Kaniwa, N., Saito, Y., Aihara, M., Matsunaga, K., Tohkin, M., Kurose, K., Sawada, J., Furuya, H., Takahashi, Y., Muramatsu, M., Kinoshita, S., Abe, M., Ikeda, H., Kashiwagi, M., Song, Y., Ueta, M., Sotozono, C., Ikezawa, Z., Hasegawa, R., 2008. HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. Pharmacogenomics 9, 1617–1622.

Kato, A., Favoreto Jr., S., Avila, P.C., Schleimer, R.P., 2007. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. The Journal of Immunology 179, 1080–1087.

Kawai, T., Akira, S., 2007. TLR signaling. Seminars in Immunology 19, 24–32.

Kawai, T., Akira, S., 2009. The roles of TLRs, RLRs and NLRs in pathogen recognition. International Immunology 21, 317–337.

Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., Ishii, K.J., Takeuchi, O., Akira, S., 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nature Immunology 6, 981–988.

Kinoshita, H., Takai, T., Le, T.A., Kamijo, S., Wang, X.L., Ushio, H., Hara, M., Kawasaki, J., Vu, A.T., Ogawa, T., Gunawan, H., Ikeda, S., Okumura, K., Ogawa, H., 2008. Cytokine milieu modulates release of thymic stromal lymphopoietin from human keratinocytes stimulated with double-stranded RNA. The Journal of Allergy and Clinical Immunology.

Kloos, W.E., Musselwhite, M.S., 1975. Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. Applied Microbiology 30, 381–385.

Klunker, S., Trautmann, A., Akdis, M., Verhagen, J., Schmid-Grendelmeier, P., Blaser, K., Akdis, C.A., 2003. A second step of chemotaxis after transendothelial migration: keratinocytes undergoing apoptosis release IFN-gamma-inducible protein 10, monokine induced by IFN-gamma, and IFN-gamma-inducible alpha-chemoattractant for T cell chemotaxis toward epidermis in atopic dermatitis. The Journal of Immunology 171, 1078–1084.

Kojima, K., Ueta, M., Hamuro, J., Hozono, Y., Kawasaki, S., Yokoi, N., Kinoshita, S., 2008. Human conjunctival epithelial cells express functional Toll-like receptor 5. The British Journal of Ophthalmology 92, 411–416.

Kumar, A., Zhang, J., Yu, F.S., 2006. Toll-like receptor 3 agonist poly(I: C)-induced antiviral response in human corneal epithelial cells. Immunology 117, 11–21.

Kumar, A., Yin, J., Zhang, J., Yu, F.S., 2007. Modulation of corneal epithelial innate immune response to pseudomonas infection by flagellin pretreatment. Investigative Ophthalmology & Visual Science 48, 4664–4670.

Kunikata, T., Yamane, H., Segi, E., Matsuoka, T., Sugimoto, Y., Tanaka, S., Tanaka, H., Nagai, H., Ichikawa, A., Narumiya, S., 2005. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. Nature Immunology 6, 524–531.

Leaute-Labreze, C., Lamireau, T., Chawki, D., Maleville, J., Taieb, A., 2000. Diagnosis, classification, and management of erythema multiforme and Stevens-Johnson syndrome. Archives of Disease in Childhood 83, 347–352.

Levi, N., Bastuji-Garin, S., Mockenhaupt, M., Roujeau, J.C., Flahault, A., Kelly, J.P., Martin, E., Kaufman, D.W., Maison, P., 2009. Medications as risk factors of Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a pooled analysis. Pediatrics 123, e297–304.

Lonjou, C., Thomas, L., Borot, N., Ledger, N., de Toma, C., LeLouet, H., Graf, E., Schumacher, M., Hovnanian, A., Mockenhaupt, M., Roujeau, J.C., 2006. A marker for Stevens-Johnson syndrome: ethnicity matters. The Pharmacogenomics Journal 6, 265–268.

Lonjou, C., Borot, N., Sekula, P., Ledger, N., Thomas, L., Halevy, S., Naldi, L., Bouwes-Bavinck, J.N., Sidoroff, A., de Toma, C., Schumacher, M., Roujeau, J.C., Hovnanian, A., Mockenhaupt, M., 2008. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. Pharmacogenet Genomics 18, 99–107.

Medzhitov, R., Preston-Hurlburt, P., Janeway Jr., C.A., 1997. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388, 394–397.

Miyairi, I., Tatireddigari, V.R., Mahdi, O.S., Rose, L.A., Belland, R.J., Lu, L., Williams, R.W., Byrne, G.I., 2007. The p47 GTPases ligp2 and Irgb10 regulate innate immunity and inflammation to murine Chlamydia psittaci infection. The Journal of Immunology 179, 1814–1824.

Mockenhaupt, M., Viboud, C., Dunant, A., Naldi, L., Halevy, S., Bouwes Bavinck, J.N., Sidoroff, A., Schneck, J., Roujeau, J.C., Flahault, A., 2008. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. The Journal of Investigative Dermatology 128, 35–44.

Mondini, M., Costa, S., Sponza, S., Gugliesi, F., Gariglio, M., Landolfo, S., 2010. The interferon-inducible HIN-200 gene family in apoptosis and inflammation: implication for autoimmunity. Autoimmunity 43, 226–231.

Mondino, B.J., Kowalski, R., 1982. Phlyctenulae and catarrhal infiltrates-occurrence in rabbits immunised with staphylococcal cell walls. Archives of Ophthalmology 100, 1968–1971.

Mondino, B.J., Brown, S.I., Rabin, B.S., 1978. Role of complement in corneal inflammation. Transactions of the Ophthalmological Societies of the United Kingdom 98, 363–366.

Mondino, B.J., Kowalski, R., Ratajczak, H.V., Petes, J., Cutler, S.B., Bronw, S.I., 1981. Rabbit model of phlyctenulosis and catarrhal infiltrates. Archives of Ophthalmology 99, 891–895.

Mondino, B.J., Brown, S.I., Biglan, A.W., 1982. HLA antigens in Stevens-Johnson syndrome with ocular involvement. Archives of Ophthalmology 100, 1453–1454.

Narumiya, S., Sugimoto, Y., Ushikubi, F., 1999. Prostanoid receptors: structures, properties, and functions. Physiol Rev 79, 1193–1226.

Neiberg, M.N., Sowka, J., 2008. Phlyctenular keratoconjunctivitis in a patient with Staphylococcal blepharitis and ocular rosacea. Optometry 79, 133–137.

Ohji, M., Ohmi, G., Kiritoshi, A., Kinoshita, S., 1987. Goblet cell density in thermal and chemical injuries. Archives of Ophthalmology 105, 1686–1688.

Ono, S.J., Abelson, M.B., 2005. Allergic conjunctivitis: update on pathophysiology and prospects for future treatment. The Journal of Allergy and Clinical Immunology 115, 118–122.

Peebles Jr., R.S., 2004. Viral infections, atopy, and asthma: is there a causal relationship? The Journal of Allergy and Clinical Immunology 113, S15–S18.

Pinna, A., Zanetti, S., Sotgiu, M., Sechi, L.A., Fadda, G., Carta, F., 1999. Identification and antibiotic susceptibility of coagulase negative staphylococci isolated in corneal/external infections. The British Journal of Ophthalmology 83, 771–773.

Power, W.J., Ghoraishi, M., Merayo-Lloves, J., Neves, R.A., Foster, C.S., 1995. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. Ophthalmology 102, 1669–1676.

Prehaud, C., Megret, F., Lafage, M., Lafon, M., 2005. Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. J Virol 79, 12893–12904.

Redfern, R.L., Reins, R.Y., McDermott, A.M., 2011. Toll-like receptor activation modulates antimicrobial peptide expression by ocular surface cells. Experimental Eye Research 92, 209–220.

Ritchie, M.D., Hahn, L.W., Roodi, N., Bailey, L.R., Dupont, W.D., Parl, F.F., Moore, J.H., 2001. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet 69, 138–147.

Roujeau, J.C., Bracq, C., Huyn, N.T., Chaussalet, E., Raffin, C., Duedari, N., 1986. HLA phenotypes and bullous cutaneous reactions to drugs. Tissue Antigens 28, 251–254.

Roujeau, J.C., Kelly, J.P., Naldi, L., Rzany, B., Stern, R.S., Anderson, T., Auquier, A., Bastuji-Garin, S., Correia, O., Locati, F., et al., 1995. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. The New England Journal of Medicine 333, 1600–1607.

Saito, H., Kubota, M., Roberts, R.W., Chi, Q., Matsunami, H., 2004. RTP family members induce functional expression of mammalian odorant receptors. Cell 119, 679–691.

Seal, D.V., McGill, J.I., Jacobs, P., Liakos, G.M., Goulding, N.J., 1985. Microbial and immunological investigations of chronic non-ulcerative blepharitis and meibomianitis. The British Journal of Ophthalmology 69, 604–611.

Shaveta, G., Shi, J., Chow, V.T., Song, J., 2010. Structural characterization reveals that viperin is a radical S-adenosyl-L-methionine (SAM) enzyme. Biochemical and Biophysical Research Communications 391, 1390–1395.

Smolin, G., Okumoto, M., 1977. Staphylococcal blepharitis. Archives of Ophthalmology 95, 812–816.

Sotozono, C., Inagaki, K., Fujita, A., Koizumi, N., Sano, Y., Inatomi, T., Kinoshita, S., 2002. Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* infections in the cornea. Cornea 21, S94–101.

Sotozono, C., Ang, L.P., Koizumi, N., Higashihara, H., Ueta, M., Inatomi, T., Yokoi, N., Kaido, M., Dogru, M., Shimazaki, J., Tsubota, K., Yamada, M., Kinoshita, S., 2007. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology 114, 1294–1302.

Sotozono, C., Ueta, M., Kinoshita, S., 2009a. The management of severe ocular complications of stevens-johnson syndrome and toxic epidermal necrolysis. Archives of Dermatology 145, 1336–1337. author reply 1337–1338.

Sotozono, C., Ueta, M., Koizumi, N., Inatomi, T., Shirakata, Y., Ikezawa, Z., Hashimoto, K., Kinoshita, S., 2009b. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. Ophthalmology 116, 685–690.

Soumelis, V., Reche, P.A., Kanzler, H., Yuan, W., Edward, G., Homey, B., Gilliet, M., Ho, S., Antonenko, S., Lauerma, A., Smith, K., Gorman, D., Zurawski, S., Abrams, J., Menon, S., McClanahan, T., de Waal-Malefyt Rd, R., Bazan, F., Kastelein, R.A., Liu, Y.J., 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nature Immunology 3, 673–680.

Stevens, A.M., Johnson, F.C., 1922. A new eruptive fever associated with stomatitis and opthalmia: report of two cases in children. American Journal of Diseases of Children 24, 526–533.

Streilein, J.W., 2003. Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nature Reviews Immunology 3, 879–889.

Strober, W., 2004. Epithelial cells pay a Toll for protection. Nature Medicine 10, 898–900.

Strober, W., Fuss, I.J., Blumberg, R.S., 2002. The immunology of mucosal models of inflammation. Annual Review of Immunology 20, 495–549.

Suzuki, T., Mitsuishi, Y., Sano, Y., Yokoi, N., Kinoshita, S., 2005. Phlyctenular keratitis associated with meibomitis in young patients. American Journal of Ophthalmology 140, 77–82.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology 33, 2233–2239.

Thygeson, P., 1969. Complications of staphylococcic blepharitis. American Journal of Ophthalmology 68, 446–449.

Tokunaga, K., Ishikawa, Y., Ogawa, A., Wang, H., Mitsunaga, S., Moriyama, S., Lin, L., Bannai, M., Watanabe, Y., Kashiwase, K., Tanaka, H., Akaza, T., Tadokoro, K.,

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Juji, T., 1997. Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. Immunogenetics 46, 199–205.
Ueta, M., 2008. Innate immunity of the ocular surface and ocular surface inflammatory disorders. Cornea 27, S31–S40.
Ueta, M., 2010. Regulation of ocular surface inflammation by prostaglandin E receptor subtype EP3. Cornea 29, S57–S61.
Ueta, M., Kinoshita, S., 2010a. Innate immunity of the ocular surface. Brain Research Bulletin 81, 219–228.
Ueta, M., Kinoshita, S., 2010b. Ocular surface inflammation mediated by innate immunity. Eye & Contact Lens 36, 269–281.
Ueta, M., Nochi, T., Jang, M.H., Park, E.J., Igarashi, O., Hino, A., Kawasaki, S., Shikina, T., Hiroi, T., Kinoshita, S., Kiyono, H., 2004. Intracellularly expressed TLR2s and TLR4s contribution to an immunosilent environment at the ocular mucosal epithelium. The Journal of Immunology 173, 3337–3347.
Ueta, M., Hamuro, J., Kiyono, H., Kinoshita, S., 2005a. Triggering of TLR3 by polyI: C in human corneal epithelial cells to induce inflammatory cytokines. Biochemical and Biophysical Research Communications 331, 285–294.
Ueta, M., Hamuro, J., Yamamoto, M., Kaseda, K., Akira, S., Kinoshita, S., 2005b. Spontaneous ocular surface inflammation and goblet cell disappearance in I kappa B zeta gene-disrupted mice. Investigative Ophthalmology & Visual Science 46, 579–588.
Ueta, M., Iida, T., Sakamoto, M., Sotozono, C., Takahashi, J., Kojima, K., Okada, K., Chen, X., Kinoshita, S., Honda, T., 2007a. Polyclonality of Staphylococcus epidermidis residing on the healthy ocular surface. Journal of Medical Microbiology 56, 77–82.
Ueta, M., Nakamura, T., Tanaka, S., Kojima, K., Kinoshita, S., 2007b. Development of eosinophilic conjunctival inflammation at late-phase reaction in mast cell-deficient mice. The Journal of Allergy and Clinical Immunology 120, 476–478.
Ueta, M., Sotozono, C., Inatomi, T., Kojima, K., Hamuro, J., Kinoshita, S., 2007c. Association of IL4R polymorphisms with stevens-johnson syndrome. The Journal of Allergy and Clinical Immunology 120, 1457–1459.
Ueta, M., Sotozono, C., Inatomi, T., Kojima, K., Tashiro, K., Hamuro, J., Kinoshita, S., 2007d. Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens-Johnson syndrome. The British Journal of Ophthalmology 91, 962–965.
Ueta, M., Sotozono, C., Tokunaga, K., Yabe, T., Kinoshita, S., 2007e. Strong association between HLA-A*0206 and stevens-johnson syndrome in the Japanese. American Journal of Ophthalmology 143, 367–368.
Ueta, M., Hamuro, J., Ueda, E., Katoh, N., Yamamoto, M., Takeda, K., Akira, S., Kinoshita, S., 2008a. Stat6-independent tissue inflammation occurs selectively on the ocular surface and perioral skin of IkappaBzeta-/- mice. Investigative Ophthalmology & Visual Science 49, 3387–3394.
Ueta, M., Sotozono, C., Inatomi, T., Kojima, K., Hamuro, J., Kinoshita, S., 2008b. Association of combined IL-13/IL-4R signaling pathway gene polymorphism with Stevens-Johnson syndrome accompanied by ocular surface complications. Investigative Ophthalmology & Visual Science 49, 1809–1813.
Ueta, M., Sotozono, C., Inatomi, T., Kojima, K., Hamuro, J., Kinoshita, S., 2008c. Association of Fas ligand gene polymorphism with stevens-johnson syndrome. The British Journal of Ophthalmology 92, 989–991.
Ueta, M., Tokunaga, K., Sotozono, C., Inatomi, T., Yabe, T., Matsushita, M., Mitsuishi, Y., Kinoshita, S., 2008d. HLA class I and II gene polymorphisms in Stevens-Johnson syndrome with ocular complications in Japanese. Molecular Vision 14, 550–555.
Ueta, M., Matsuoka, T., Narumiya, S., Kinoshita, S., 2009a. Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. The Journal of Allergy and Clinical Immunology 123, 466–471.
Ueta, M., Sotozono, C., Takahashi, J., Kojima, K., Kinoshita, S., 2009b. Examination of *Staphylococcus aureus* on the ocular surface of patients with catarrhal ulcers. Cornea 28, 780–782.
Ueta, M., Uematsu, S., Akira, S., Kinoshita, S., 2009c. Toll-like receptor 3 enhances late-phase reaction of experimental allergic conjunctivitis. The Journal of Allergy and Clinical Immunology 123, 1187–1189.
Ueta, M., Hamuro, J., Kinoshita, S., 2010a. Spatio-temporal dual effects of IkappaBzeta dictates the caution on visual disturbance resulting from IkappaBzeta deficiency. Immunology Letters 133, 115.
Ueta, M., Mizushima, K., Yokoi, N., Naito, Y., Kinoshita, S., 2010b. Gene-expression analysis of polyI: C-stimulated primary human conjunctival epithelial cells. The British Journal of Ophthalmology 94, 1528–1532.
Ueta, M., Sotozono, C., Nakano, M., Taniguchi, T., Yagi, T., Tokuda, Y., Fuwa, M., Inatomi, T., Yokoi, N., Tashiro, K., Kinoshita, S., 2010c. Association between prostaglandin E receptor 3 polymorphisms and Stevens-Johnson syndrome identified by means of a genome-wide association study. The Journal of Allergy and Clinical Immunology 126, 1218–1225. e1210.
Ueta, M., Kawai, T., Yokoi, N., Akira, S., Kinoshita, S., 2011a. Contribution of IPS-1 to polyI: C-induced cytokine production in conjunctival epithelial cells. Biochemical and Biophysical Research Communications 404, 419–423.
Ueta, M., Matsuoka, T., Yokoi, N., Kinoshita, S., 2011b. Prostaglandin E2 suppresses polyinosine-polycytidylic acid (polyI: C)-stimulated cytokine production via prostaglandin E2 receptor (EP) 2 and 3 in human conjunctival epithelial cells. The British Journal of Ophthalmology 95, 859–863.
Ueta, M., Matsuoka, T., Yokoi, N., Kinoshita, S., 2011c. Prostaglandin E receptor subtype EP3 downregulates TSLP expression in human conjunctival epithelium. The British Journal of Ophthalmology 95, 742–743.
Ueta, M., Sotozono, C., Yokoi, N., Inatomi, T., Kinoshita, S., 2011d. Prostaglandin E receptor subtype EP3 expression in human conjunctival epithelium and its changes in various ocular surface disorders. PloS One 6, e25209.
Ueta, M., Sotozono, C., Yokoi, N., Kinoshita, S., 2012a. Downregulation of monocyte chemoattractant protein 1 expression by prostaglandin E(2) in human ocular surface epithelium. Archives of Ophthalmology 130, 249–251.
Ueta, M., Tamiya, G., Tokunaga, K., Sotozono, C., Ueki, M., Sawai, H., Inatomi, T., Matsuoka, T., Akira, S., Narumiya, S., Tashiro, K., Kinoshita, S., 2012b. Epistatic interaction between Toll-like receptor 3 (TLR3) and prostaglandin E receptor 3 (PTGER3) genes. The Journal of Allergy and Clinical Immunology 129, 1413–1416.
Vane, J.R., Bakhle, Y.S., Botting, R.M., 1998. Cyclooxygenases 1 and 2. Annual Review of Pharmacology and Toxicology 38, 97–120.
Wolf, R., Orion, E., Marcos, B., Matz, H., 2005. Life-threatening acute adverse cutaneous drug reactions. Clinics in Dermatology 23, 171–181.
Xu, Y., Johansson, M., Karlsson, A., 2008. Human UMP-CMP kinase 2, a novel nucleoside monophosphate kinase localized in mitochondria. The Journal of Biological Chemistry 283, 1563–1571.
Yamamoto, M., Yamazaki, S., Uematsu, S., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Kuwata, H., Takeuchi, O., Takeshige, K., Saitoh, T., Yamaoka, S., Yamamoto, N., Yamamoto, S., Muta, T., Takeda, K., Akira, S., 2004. Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaB-zeta. Nature 430, 218–222.
Yamane, Y., Aihara, M., Ikezawa, Z., 2007. Analysis of Stevens-Johnson syndrome and toxic epidermal necrolysis in Japan from 2000 to 2006. Allergology International 56, 419–425.
Yamazaki, S., Muta, T., Takeshige, K., 2001. A novel IkappaB protein, IkappaB-zeta, induced by proinflammatory stimuli, negatively regulates nuclear factor-kappaB in the nuclei. The Journal of Biological Chemistry 276, 27657–27662.
Yetiv, J.Z., Bianchine, J.R., Owen Jr., J.A., 1980. Etiologic factors of the stevens-johnson syndrome. Southern Medical Journal 73, 599–602.
Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Mallett, K., Cousins, D., Robinson, D., Zhang, G., Zhao, J., Lee, T.H., Corrigan, C., 2005. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. The Journal of Immunology 174, 8183–8190.
Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Fang, C., Cousins, D., Zhang, G., Gu, S., Gao, Z., Shamji, B., Edwards, M.J., Lee, T.H., Corrigan, C.J., 2008. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. The Journal of Immunology 181, 2790–2798.
Zhang, J., Xu, K., Ambati, B., Yu, F.S., 2003. Toll-like receptor 5-mediated corneal epithelial inflammatory responses to Pseudomonas aeruginosa flagellin. Investigative ophthalmology & visual science 44, 4247–4254.
Zhang, D., Zhang, G., Hayden, M.S., Greenblatt, M.B., Bussey, C., Flavell, R.A., Ghosh, S., 2004. A toll-like receptor that prevents infection by uropathogenic bacteria. Science 303, 1522–1526.
Zhang, J., Kumar, A., Wheater, M., Yu, F.S., 2008. Lack of MD-2 expression in human corneal epithelial cells is an underlying mechanism of lipopolysaccharide (LPS) unresponsiveness. Immunology and Cell Biology 87, 141–148.
Ziegler, S.F., Liu, Y.J., 2006. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. Nature Immunology 7, 709–714.

Abbreviation

PFGE: pulsed-field gel electrophoresis
TLRs: Toll-like receptors
IFN: interferon
IL: interleukin
TNF: tumor necrosis factor
PGN: peptidoglycan
ds: double-stranded
polyI:C: polyinosine-polycytidylic acid
LPS: Lipopolysaccharide
CpG: deoxy-cytidylate-phosphate-deoxy-guanylate
RT-PCR: reverse transcription-polymerase chain reaction
IP-10: IFN-gamma inducible protein 10
NLRs: nucleotide-binding oligomerization domain (NOD)-like receptors
RLRs: retinoic acid-inducible gene-I (RIG-I)-like receptors
Mx2: myxovirus (influenza virus) resistance 2
Rsad2: radical S-adenosyl methionine domain containing 2
Cmpk2: cytidine monophosphate (UMP-CMP) kinase 2
Cxcl10: chemokine (C-X-C motif) ligand 10
Mx1: myxovirus (influenza virus) resistance 1
Ifi44: interferon-induced protein 44
Ifi203: interferon-activated gene 203
Iigp2: interferon-inducible GTPase 2
Rtp4: receptor transporter protein 4
TSLP: thymic stromal lymphopoietin
PG: prostaglandin
TX: thromboxane
SJS: Stevens-Johnson syndrome
TEN: toxic epidermal necrolysis
NSAIDs: non-steroidal anti-inflammatory drugs

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RESEARCH LETTERS

Downregulation of Monocyte Chemoattractant Protein 1 Expression by Prostaglandin E_2 in Human Ocular Surface Epithelium

Elsewhere, we reported that in the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of interleukin 6 (IL-6), IL-8, and monocyte chemoattractant protein 1 (MCP-1) were dramatically increased.[1] We also reported that Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications was associated with polymorphism of the prostaglandin E receptor 3 (EP_3) gene (*PTGER3*).[2]

Prostanoids are a group of lipid mediators that form in response to various stimuli. They include prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2. There are 4 subtypes of the PGE receptor: EP_1, EP_2, EP_3, and EP_4. We previously reported that PGE_2 suppresses polyinosine–polycytidylic acid (polyI:C)–stimulated cytokine production via EP_2 and/or EP_3 in human ocular surface epithelial cells.[3,4] PolyI:C is a ligand of Toll-like receptor 3, which is strongly expressed in ocular surface epithelium.[5] We found that PGE_2 suppresses the production of IL-6, chemokine (C-X-C motif) ligand 10, chemokine (C-X-C motif) ligand 11, and chemokine (C-C motif) ligand 5 but not IL-8 by epithelial cells on the human ocular surface[3]; it remains to be determined whether it also suppresses MCP-1 production. Monocyte chemoattractant protein 1 plays a significant role in the recruitment of monocytes and lymphocytes to the site of cellular immune reactions. In this study, we investigated whether PGE_2 downregulates polyI:C-induced MCP-1 production.

All experiments were conducted in accordance with the principles set forth in the Declaration of Helsinki. Enzyme-linked immunosorbent assay and quantitative real-time polymerase chain reaction were performed with primary human conjunctival epithelial cells and immortalized human corneal-limbal epithelial cells using previously described methods (eAppendix, http://www.archophthalmol.com).[3]

First, we examined whether PGE_2 downregulated the production and messenger RNA (mRNA) expression of MCP-1 induced by polyI:C stimulation in human conjunctival and corneal epithelial cells. We found that it significantly attenuated the production of MCP-1 (**Figure**, A). Quantitative real-time polymerase chain reaction confirmed that the mRNA expression of MCP-1 was significantly downregulated by PGE_2 (Figure, A).

Next, we examined which PGE_2 receptor(s) contributed to the downregulation of polyI:C-induced MCP-1. We used the EP_2 agonist ONO-AE-259, the EP_3 agonist ONO-AE-248, and the EP_4 agonist ONO-AE-329. Enzyme-linked immunosorbent assay showed that the EP_2 and EP_3 agonists significantly suppressed the polyI:C-induced production of MCP-1, while the EP_4 agonist did not exert suppression (Figure, B). Quantitative real-time polymerase chain reaction confirmed that the EP_2 and EP_3 agonists significantly downregulated the mRNA expression of MCP-1 (Figure, C). Thus, our results document that PGE_2 attenuated the mRNA expression and production of MCP-1 via both EP_2 and EP_3.

In human macrophages, PGE_2 attenuated the lipopolysaccharide-induced mRNA and protein expression of chemokines including MCP-1 through EP_4.[6] On the other hand, we demonstrated that in human ocular surface epithelial cells, PGE_2 attenuated the polyI:C-induced mRNA and protein expression of MCP-1 through EP_2 and EP_3 but not EP_4. Our findings suggest that EP_2 and EP_3 play important roles in the regulation of inflammation in epithelial cells, while EP_2 and EP_4 have important roles in immune cells such as macrophages.

In the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of IL-6, IL-8, and MCP-1 were dramatically increased.[1] Although IL-8 was not regulated by PGE_2, IL-6 was regulated by PGE_2 via EP_3 in human ocular surface epithelial cells.[3] Herein, we demonstrated that MCP-1 could be regulated by PGE_2 via EP_2 and EP_3. The regulation of cytokine production by PGE_2 may be associated with the pathogenesis of Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications because it was associated with polymorphism of the EP_3 gene (*PTGER3*), one of the PGE receptors (EP_1, EP_2, EP_3, EP_4).[2]

In summary, our results show that MCP-1 produced by human ocular surface epithelial cells could be downregulated by PGE_2 via EP_2 and EP_3.

Mayumi Ueta, MD, PhD
Chie Sotozono, MD, PhD
Norihiko Yokoi, MD, PhD
Shigeru Kinoshita, MD, PhD

Author Affiliations: Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University (Dr Ueta) and Department of Ophthalmology, Kyoto Prefectural University of Medicine (Drs Ueta, Sotozono, Yokoi, and Kinoshita), Kyoto, Japan.
Correspondence: Dr Ueta, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Ka-

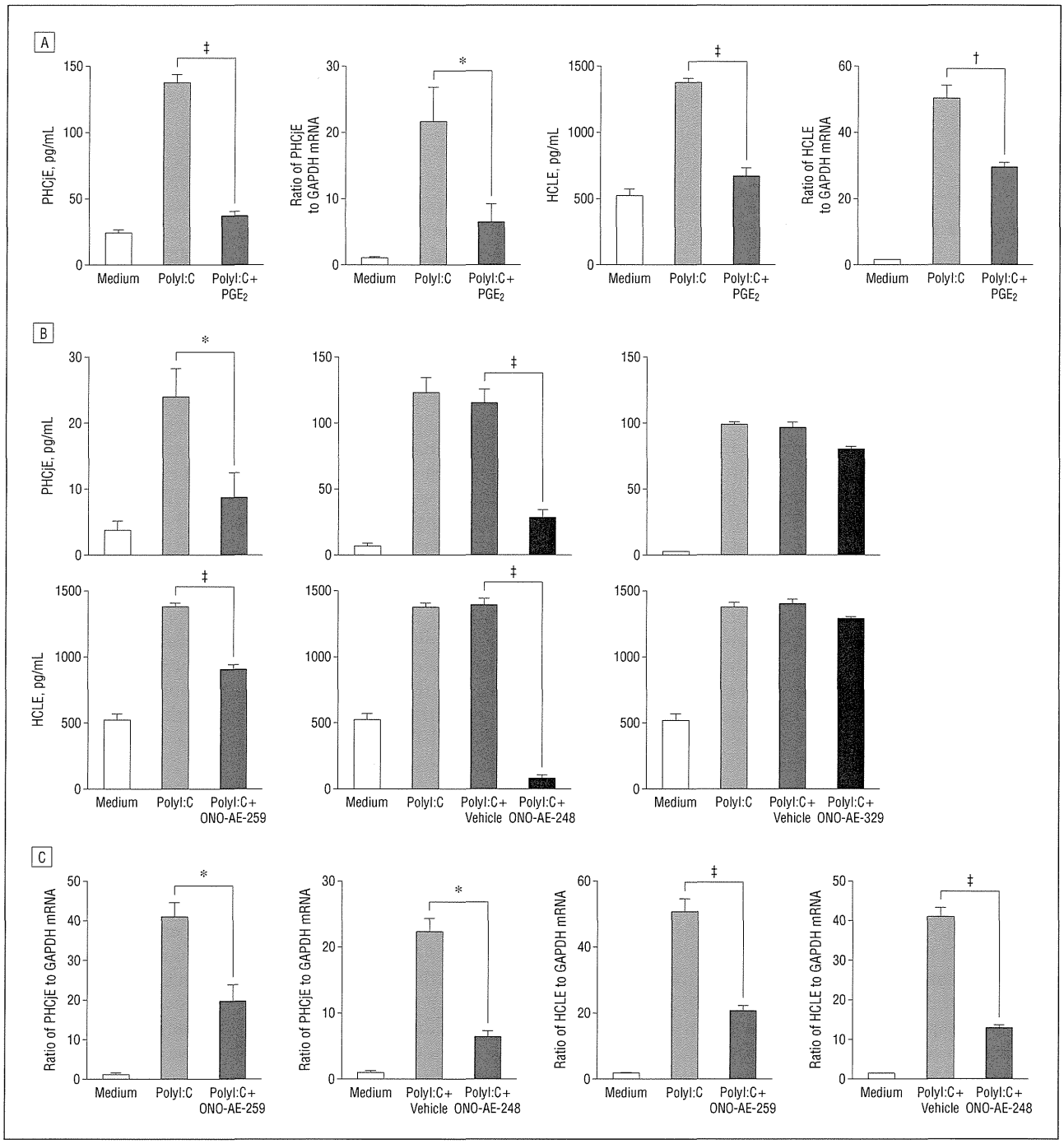

Figure. Prostaglandin E_2 (PGE_2) attenuated the messenger RNA (mRNA) expression and production of monocyte chemoattractant protein 1 via both prostaglandin E receptor 2 (EP_2) and EP_3. A, Primary human conjunctival epithelial cells (PHCjE) and human corneal-limbal epithelial cells (HCLE) were exposed to 10 μg/mL of polyinosine–polycytidylic acid (polyI:C) and 100 μg/mL of PGE_2 for 24 hours (enzyme-linked immunosorbent assay) or 6 hours (quantitative real-time polymerase chain reaction). GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase. B and C, The PHCjE and HCLE were exposed to 10 μg/mL of polyI:C and 10 μg/mL of the EP_2, EP_3, or EP_4 agonist for 24 hours (enzyme-linked immunosorbent assay) (B) or 6 hours (quantitative real-time polymerase chain reaction) (C). Data are representative of 3 separate experiments and are given as the mean (SEM) from 1 experiment carried out in 6 to 8 wells (enzyme-linked immunosorbent assay) (B) or 4 to 6 wells (quantitative real-time polymerase chain reaction) (C) per group. $^*P<.05$; $\dagger P<.005$; $\ddagger P<.001$.

jiicho, Hirokoji, Kawaramachi, Kamigyoku, Kyoto 602-0841, Japan (mueta@koto.kpu-m.ac.jp).

Author Contributions: Dr Ueta had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Online-Only Material: The eAppendix is available at http://www.archophthalmol.com.
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1. Yagi T, Sotozono C, Tanaka M, et al. Cytokine storm arising on the ocular surface in a patient with Stevens-Johnson syndrome. *Br J Ophthalmol*. 2011; 95(7):1030-1031.
2. Ueta M, Sotozono C, Nakano M, 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(6): 1218-1225, e10.
3. 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(6):859-863.
4. 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(5):742-743.
5. Ueta M, Kinoshita S. Innate immunity of the ocular surface. *Brain Res Bull*. 2010;81(2-3):219-228.
6. Takayama K, García-Cardena G, Sukhova GK, Comander J, Gimbrone MA Jr, Libby P. Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. *J Biol Chem*. 2002;277(46):44147-44154.

Depth Profile Study of Abnormal Collagen Orientation in Keratoconus Corneas

In a previous study,[1] we used femtosecond laser technology to cut ex vivo human corneas into anterior, mid, and posterior sections, after which x-ray scatter patterns were obtained at fine intervals over each specimen. Data analysis revealed the predominant orientation of collagen at each sampling site, which was assembled to show the variation in collagen orientation between central and peripheral regions of the cornea and as a function of tissue depth. We hypothesized that the predominantly orthogonal arrangement of collagen (directed toward opposing sets of rectus muscles) in the mid and posterior stroma may help to distribute strain in the cornea by allowing it to withstand the pull of the extraocular muscles. It was also suggested that the more isotropic arrangement in the anterior stroma may play a role in tissue biomechanics by resisting intraocular pressure while at the same time maintaining corneal curvature. This article, in conjunction with our findings of abnormal collagen orientation in full-thickness keratoconus corneas,[2,3] received a great deal of interest from the scientific community and prompted the following question: how does collagen orientation change as a function of tissue depth when the anterior curvature of the cornea is abnormal, as in keratoconus? Herein, we report findings from our investigation aimed at answering this question.

Methods. The Baron chamber used in our previous study[1] was adapted to enable corneal buttons to be clamped in place and inflated (by pumping physiological saline into the posterior compartment) to restore their natural curvature. A button diameter of 8 mm or larger was deemed necessary to ensure tissue stability during this process.

The next step, obtaining fresh, full-thickness, keratoconus buttons of sufficient diameter, proved to be problematic owing to the increasing popularity of deep anterior lamellar keratoplasty. Recently, however, the opportunity arose to examine an 8-mm full-thickness (300-340 µm minus epithelium) keratoconus corneal button with some central scarring and a mean power greater than 51.8 diopters (**Figure 1**). The tissue was obtained in accordance with the tenets of the Declaration of Helsinki and with full informed consent from a 31-year-old patient at the time of penetrating keratoplasty. Using techniques detailed previously,[1] the corneal button was clamped in the chamber and inflated. The central 6.3-mm region of the button was then flattened by the applanation cone and a single cut was made at a depth of 150 µm from the surface using an IntraLase 60-kHz femtosecond laser (Abbott Medical Optics Inc),[1] thus splitting the cornea into anterior and posterior sections of roughly equal thickness. Wide-angle x-ray scattering patterns were collected at 0.25-mm intervals over each cor-

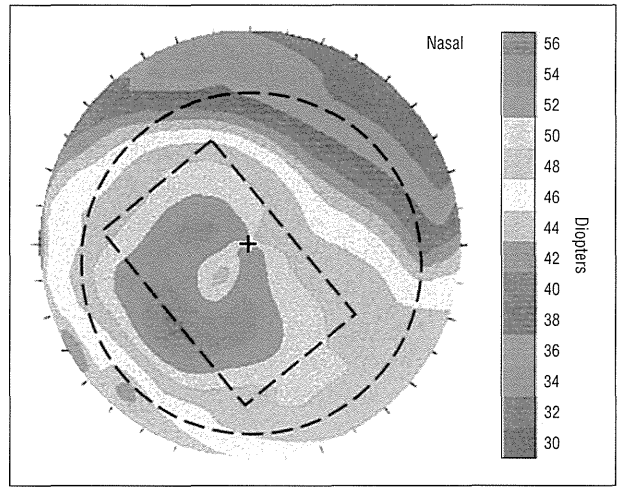

Figure 1. Corneal topography of the keratoconus cornea (recorded 12 years previously).[3] The broken lines show the 6.3-mm region of the cornea cut with the femtosecond laser (circle) and the region of greatest corneal steepening depicted in Figure 2 (rectangle).

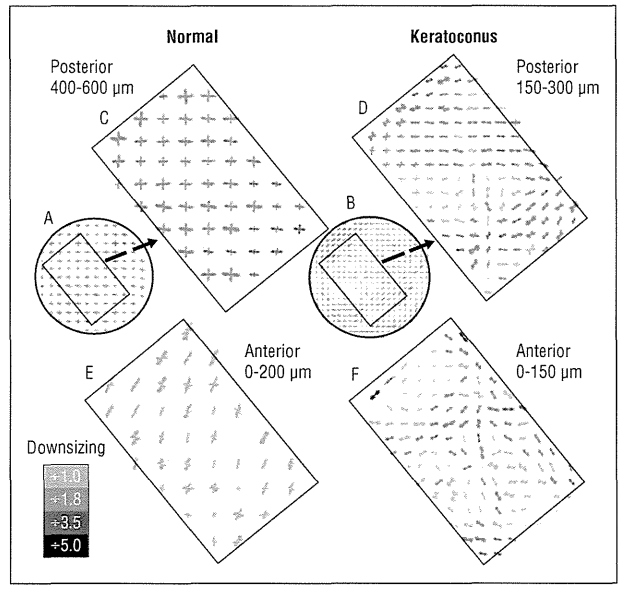

Figure 2. Collagen orientation in the normal (A) and keratoconus (B) posterior stroma (central 6.3 mm). The highlighted regions of the posterior (C and D) and anterior (E and F) stroma are expanded. Large vector plots showing high collagen alignment are downsized (key).