Two cases of dupilumab-associated conjunctivitis with high expression of IL-8 mRNA on the ocular surface: a case report

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Case report

Keywords: dupilumab, conjunctivitis, eotaxin-2, IL-8

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Abstract

Background: Dupilumab-induced ocular surface disease (DIOSD) has been reported in patients with atopic dermatitis treated with dupilumab, and has been recognized as an adverse event of dupilumab. Our objective was to describe two cases of DIOSD with alterations in eotaxin-2 and interleukin (IL)-8 messenger ribonucleic acid (mRNA) expression on the ocular surface.

Case presentation: In the ocular surface test, specimens were collected from the patient's ocular surface, and eotaxin-2 and IL-8 mRNA levels in the specimens were measured using real-time polymerase chain reaction. The clinical score of ocular surface findings was quantified using a 5-5-5 exacerbation grading scale for allergic conjunctivitis. The first case was of a 27-year-old man who developed DIOSD 3 months after starting treatment with dupilumab injection for atopic dermatitis. After 5 weeks of topical instillation of tacrolimus ophthalmic suspension, the clinical score of ocular surface findings improved and IL-8 and eotaxin-2 mRNA expression levels gradually decreased. The second patient was a 55-year-old man who developed DIOSD 11 weeks after the start of treatment with dupilumab injection for atopic dermatitis. Four weeks after starting

ophthalmological treatment with tacrolimus ophthalmic suspension, his clinical scores on ocular surface findings improved and IL-8 mRNA expression levels decreased. The ocular surface test in this case revealed increased expression levels of IL-8 mRNA on the ocular surface at the onset of DIOSD, which decreased with the improvement of objective findings.

Conclusions: DIOSD, which has been successfully treated with tacrolimus ophthalmic suspension, may involve IL-8-related inflammation in addition to type 2 inflammation.

Keywords: dupilumab, conjunctivitis, eotaxin-2, IL-8

Background

Atopic dermatitis (AD) is a chronic inflammatory disease characterized by severe skin irritation and pruritic, erythematous, and scarring skin lesions. The major immunological pathogenesis of AD is understood to be the T helper type 2 (Th2) response, including the cytokine effects of interleukin (IL)-4 and IL-13, with additional roles for Th17, Th22, and Th1 cytokines in certain disease subtypes. The cytokines produced by

ILC2, including IL-13, are also involved in the immunological pathogenesis of AD. Ocular complications such as atopic keratoconjunctivitis (AKC), keratoconus, cataracts, and retinal detachment are known to develop in severe AD.

Dupilumab, an anti-human alpha subunit of IL-4 and IL-13 receptor monoclonal antibody, is the only dual inhibitor of IL-4 and IL-13 signaling. In addition, dupilumab therapy has been approved for indications including atopic dermatitis, bronchial asthma, and rhinosinusitis with nasal polyposis in Japan, and reduced Th2 responses in these allergic diseases [1]. Conjunctivitis, blepharitis, and keratitis have been reported in patients with AD treated with dupilumab, and have been recognized as adverse events of dupilumab [2]. Dupilumab-induced blepharitis and conjunctivitis have a wide variety of clinical phenotypes including moderate to severe conjunctivitis [3], follicular conjunctivitis [4], giant papillary conjunctivitis [5, 6], blepharoconjunctivitis [7], cicatrizing conjunctivitis [8], and corneal limbitis [4]. Therefore, ocular surface diseases that occur during dupilumab treatment are called dupilumab-induced ocular surface disease (DIOSD) [9, 10]. However, the detailed pathogenesis of DIOSD is not yet fully understood.

We reported the usefulness of clinical scores based on objective findings [11] and ocular surface test using messenger ribonucleic acid (mRNA) expression levels of allergic inflammatory factors including cytokines, chemokines, and eosinophil-associated factors to evaluate the severity of vernal and atopic keratoconjunctivitis [12, 13]. In this study, IL-8 as a neutrophil-related factor [14] and eotaxin-2/CCL24 as an eosinophil-related factor [15] were used as markers of ocular surface test. In addition, to analyze the pathogenesis of inflammation occurring in DIOSD, we conducted an observational study using clinical scores and ocular surface test for DIOSD in patients with atopic dermatitis undergoing treatment with dupilumab.

Case presentation

Clinical severity score

To evaluate the clinical severity score, our recently reported 5-5-5 exacerbation grading scale for allergic conjunctival diseases [11] was used. This clinical severity score was based on the presence of five findings (active giant papillae, gelatinous infiltrates of the limbus, exfoliative epithelial keratopathy, shield ulcer, papillary proliferation at lower

palpebral conjunctiva) that scored 100 points, five findings (blepharitis, papillary proliferation with velvety appearance, Horner–Trantas spots, edema of bulbal conjunctiva, superficial punctate keratopathy) that scored 10 points, and five findings (papillary at upper palpebral conjunctiva, follicular lesion at lower palpebral conjunctiva, hyperemia of palpebral conjunctiva, hyperemia of bulbal conjunctiva, and lacrimal effusion) that scored 1 point. In addition, the Eczema Area Severity Index (EASI) [16] and Patient-Oriented Eczema Measure (POEM) scores [17] were used to evaluate the severity and activity of AD.

Ocular surface test

The ocular surface test is an ophthalmological clinical test that combines specimen collection by impression cytology and the measurement of cytokine and chemokine mRNA levels expressed on the ocular surface by quantitative reverse transcription polymerase chain reaction (qRT-PCR) [12]. This study was approved by the Institutional Review Board of Nihon University School of Medicine (approval number: RK-190709-2), and written informed consent was obtained from all patients prior to testing.

Impression cytology

Using the impression cytology method, specimens for qRT-PCR were collected from the upper palpebral conjunctiva. Specimens were collected by pressing a filter paper disk made by excising the tip of a Schirmer test paper (Tear Production Measuring Strips; AYUMI Pharmaceutical Corporation, Tokyo, Japan) against the unanesthetized palpebral conjunctiva. Messenger RNA (mRNA) was extracted from the filter paper disc using a MagLEAD® automated nucleic acid extraction system (Precision System Science, Chiba, Japan).

Real-time reverse transcription polymerase chain reaction

Real-time RT-PCR was performed using the GeneSoc® microfluidic real-time PCR system (KYORIN Pharmaceutical, Tokyo, Japan), TaqMan gene expression assay (Life Technologies), and predesigned primers/probes, including Hs99999034_m1 (IL-8) and Hs00171082_m1 (eotaxin-2) (Life Technologies Japan, Tokyo, Japan). The target cycle threshold (Ct) values were normalized to those of GAPDH (Hs99999905_m1) from the same sample. The relative expression levels of each target gene were determined using the ΔΔCT method. The reference value for each mRNA expression level was set as 1.

Case series

Case 1

A 27-year-old man had moderate-severe AD (EASI score was 27.7 points at the beginning of dupilumab treatment), presented to ophthalmology department of our hospital complaining progressively increasing hyperemia, blepharitis, and epiphora in his bilateral eyes 3 months after starting dupilumab treatment for AD (day 0). At the initial visit to our department, her EASI score was 3.2 points. Slit-lamp examination revealed atopic blepharitis, velvety papillary proliferation of the upper palpebral conjunctiva, and severe hyperemia of the bulbar conjunctiva. The clinical severity score at the initial visit was 134. He was diagnosed with DIOSD and treated with 0.1% tacrolimus hydrate ointment once per day for atopic blepharitis, tacrolimus ophthalmic suspension twice per day, and 0.5% cefmenoxime ophthalmic solution twice per day for conjunctivitis. Five weeks after starting ophthalmological treatment (week 5), the objective findings of blepharitis and conjunctivitis improved (Figure 1), and the clinical severity score decreased to 13 points. The results of the clinical severity scores and ocular surface tests

129 are shown in Table 1 and Figure 2. An ocular surface test at the initial ophthalmology
130 visit showed markedly elevated IL-8 mRNA levels in both eyes. At week 5, the IL-8
131 mRNA levels had decreased more than 50-fold. The eotaxin-2 mRNA levels showed a
132 similar trend in the ocular surface tests, but the changes during the treatment period were
133 less pronounced.
134
135 *Case 2*
136 A 55-year-old man with severe AD (EASI score of 41.2 points) developed AKC and
137 continued AKC treatment in the ophthalmology department of our hospital. He was
138 started on dupilumab treatment by a dermatologist owing to the severity and
139 refractoriness of his AD. Before dupilumab administration, his clinical scores were
140 recorded, and specimens were collected from both eyes for the ocular surface test. The
141 clinical severity score was determined to be 113 points. Eleven weeks after the start of
142 dupilumab treatment (day 0), conjunctival hyperemia exacerbated. His objective findings
143 in the lid, conjunctiva, and cornea included atopic blepharitis, velvety papillary
144 proliferation of the upper palpebral conjunctiva predominantly in the right eye, and severe

145 hyperemia of the bulbal conjunctiva (Figure 3). His clinical severity score in both eyes
146 increased to 124 points. He was diagnosed with DIOSD, and treatment for DIOSD was
147 continued with tacrolimus ointment once a day, which was changed from dexamethasone
148 ointment for atopic blepharitis and tacrolimus ophthalmic solution twice a day for AKC
149 and DIOSD. Four weeks after starting ophthalmological treatment (week 4), the clinical
150 severity score improved to 24 points. The alterations in EASI, POEM, clinical severity
151 score, and ocular surface test results are shown in Table 1 and Figure 4. At the first
152 ophthalmologic visit after the onset of conjunctivitis, the ocular surface test showed a
153 markedly higher level of IL-8 mRNA compared to baseline in both eyes. At week 4, IL-
154 8 mRNA levels had decreased. The eotaxin-2 mRNA levels were generally low in the
155 ocular surface tests, although mild changes were noted during the course of the treatment.
156

157 **Discussion and Conclusions**

158 In our case report on DIOSD, we clarified the clinical characteristics of AD patients
159 with DIOSD using clinical scores and ocular surface tests. Each patient showed
160 blepharitis and severe conjunctivitis was commonly observed, and IL-8 mRNA

161 expression was increased on the ocular surface during the development of DIOSD.
162 Furthermore, ophthalmic treatment with a tacrolimus ophthalmic suspension and
163 tacrolimus ointment is useful for DIOSD.

164 The 5-5-5 exacerbation grading scale for allergic conjunctivitis (clinical severity
165 score) was used to evaluate the ophthalmological subjective findings in this study. In
166 addition, the clinical severity score can quantify and evaluate the clinical findings
167 associated with conjunctivitis [11]. For patients with AKC, the severe stage was set at
168 more than 100 points, moderate stage was set between 30 and 100 points, and mild stage
169 was set at less than 30 points. In the present case, the clinical score was > 100 points, and
170 the conjunctivitis that developed during dupilumab treatment was judged to be at a severe
171 stage. The clinical findings of DIOSD in our cases were characterized by severe
172 conjunctival hyperemia with conjunctival swelling in the palpebral and bulbar
173 conjunctiva, which was the same as previously reported [3, 4, 9, 10]. However, these
174 conjunctival findings are not specific to DIOSD, and the differential diagnosis of acute
175 exacerbation of AKC is difficult. In the future, specific findings that can be used for a
176 definitive diagnosis of DIOSD should be established.

177 The results of the ocular surface test in our case revealed increased IL-8 mRNA
178 expression levels in the upper tarsal conjunctiva at the onset of DIOSD. IL-8 is a CXC
179 chemokine involved in neutrophil migration, and the IL-8 mRNA expression level was
180 used in this study as an ocular surface marker of neutrophilic inflammation. Increased IL-
181 8 levels in the tears have been reported in patients with infectious conjunctivitis and
182 trauma [18]. Furthermore, in allergic conjunctival diseases, IL-8 levels have also been
183 reported to be elevated in the tears of patients with giant papillary conjunctivitis and
184 vernal keratoconjunctivitis (VKC) [19]. Aso et al. reported that expression levels of IL-
185 1α, IL-8, IL-16, and eotaxin-2 mRNA were elevated in the ocular surface test of patients
186 with chronic ACD, including AKC and VKC, and that there was a significant correlation
187 between IL-1α and IL-8, and between IL-16 and eotaxin-2. We speculated that two
188 inflammatory systems, eotaxin-2-associated inflammation and IL-8-associated
189 inflammation, are involved in the pathogenesis of chronic allergic conjunctivitis,
190 including AKC and VKC [13]. In addition, Leonaldi et al. demonstrated that ocular
191 surface tests of patients with VKC showed increased mRNA expression of Th2/Th17-
192 signaling families and proinflammatory cytokines [20], suggesting that Th2 and Th17

193 reactions are key factors in the pathogenesis of chronic allergic diseases. The increase in
194 IL-8 levels in inflammatory tissues is known to be related to innate immunity, Th17
195 response, histamine stimulation, and reactive oxygen species [21]. It is unclear what
196 triggered the increased IL-8 in our patients, but it is possible that DIOSD causes a
197 different inflammatory response than eosinophilic inflammation induced by Th2
198 inflammation. Bakker et al. reported that conjunctival biopsies from patients with atopic
199 dermatitis treated with dupilumab showed elevated Th1/Th17 cytokines in the
200 conjunctiva [22].

201 In contrast, eotaxin-2 mRNA expression, which is used as a marker of eosinophilic
202 inflammation, remained mildly increased during the observation period. These test results
203 indicate that dupilumab injections and tacrolimus eye drop instillation suppress Th2
204 reactions and eosinophilic inflammation in the conjunctival tissues.

205 Our case report had several limitations. First, the diagnostic criteria for DIOSD are
206 unclear. In the future, it will be necessary to establish diagnostic criteria for DIOSD in a
207 larger number of patients with AD receiving dupilumab treatment. Second, the clinical
208 tests for ocular surface markers were semi-quantitative. In the future, the results of ocular

surface tests should be incorporated into diagnostic criteria as absolute measurements.

In conclusion, DIOSD, which has been successfully treated with tacrolimus ophthalmic suspension, may involve IL-8-related inflammation in addition to type 2 inflammation.

Abbreviations

ACD, allergic conjunctival disease; AD, atopic dermatitis; AKC, atopic keratoconjunctivitis; DIOSD, Dupilumab-induced ocular surface disease; EASI, Eczema Area Severity Index; IL-8, interleukin-8; POEM, Patient-Oriented Eczema Measure; RT-PCR, reverse transcription polymerase chain reaction; VKC, vernal keratoconjunctivitis

Declarations

Ethics approval and consent to participate

The ocular surface test was approved by the Institutional Review Board of Nihon University School of Medicine, and written informed consent was obtained from all patients prior to testing.

Consent for publication

Informed consent for the publication of this case report was obtained from all patients.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

J.S. received honoraria from Santen Pharmaceutical Co., Ltd. and Senju Pharmaceutical Co., Ltd. The authors declare that they have no conflicts of interest.

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No formal funding was obtained for this study.

Authors' contributions

RA and JS engaged in background research on the subject, collection of patient data, and

data analysis, and were the major contributors in writing the manuscript. AH, AT, YT, and NI collected patient data and analyzed the data. SY oversaw the study and reviewed the manuscript. All authors have read and approved the final manuscript.

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Table 1. Clinical scores of 5-5-5 exacerbation grading scale, EASI, and POEM

Case	Visit	5-5-5 exacerbation grading scale (points)		EASI score (points)	POEM score (points)
		Right eye	Left eye		
Patient 1	Start of dupilumab injection	NT	NT	27.7	23
	Week 18 (Day 0*)	134	134	3.2	12
	Week 23	13	13	4	9
Patient 2	Start of dupilumab injection	14	14	41.2	28
	Week 11 (Day 0*)	124	124	5.65	1
	Week 15	24	24	4.1	0

*: Day 0 means the first visit to ophthalmologist after the onset of conjunctivitis.

NT, not tested

Figure Legends

Figure 1. Photographs of blepharitis and conjunctivitis in case 1

A. At the initial ophthalmology visit, posterior blephalitis including meibomian gland inflammation, conjunctivitis with velvety appearance of palpebral conjunctivitis, severe bulbal hyperemia, and mucopurulent discharge were observed.

B. Five weeks after starting ophthalmologic treatment, clinical findings of blepharoconjunctivitis were improved.

Figure 2. Results of ocular surface test for IL-8 and eotaxin-2 in case 1

A. Relative expression of IL-8 mRNA on the ocular surface. IL-8 mRNA levels peaked in both eyes at the onset of conjunctivitis (day 0) and decreased as conjunctivitis became milder.

B. Relative expression levels of eotaxin-2 mRNA on the ocular surface. Eotaxin-2 mRNA levels were mildly elevated at the onset of conjunctivitis.

NT, not tested.

342

343 Figure 3. Photographs of blepharitis and conjunctivitis in case 2

344 A. At the first ophthalmology visit after the onset of conjunctivitis, conjunctivitis
345 with a velvety appearance of palpebral conjunctivitis and severe bulbal hyperemia
346 were observed.

347 B. Nine weeks after starting ophthalmologic treatment, the clinical findings of
348 conjunctivitis mostly resolved, but mild hyperemia continued.

349

350 Figure 4. Results of ocular surface test for IL-8 and eotaxin-2 in case 2.

351 A. Relative expression of IL-8 mRNA on the ocular surface. The IL-8 mRNA
352 levels peaked in both eyes at the onset of conjunctivitis (day 0) and decreased
353 thereafter.

354 B. Relative expression levels of eotaxin-2 mRNA on the ocular surface. Eotaxin-
355 2 mRNA levels were mildly elevated in (right eye) or virtually unchanged (left
356 eye) with the onset of conjunctivitis.

357

Figures

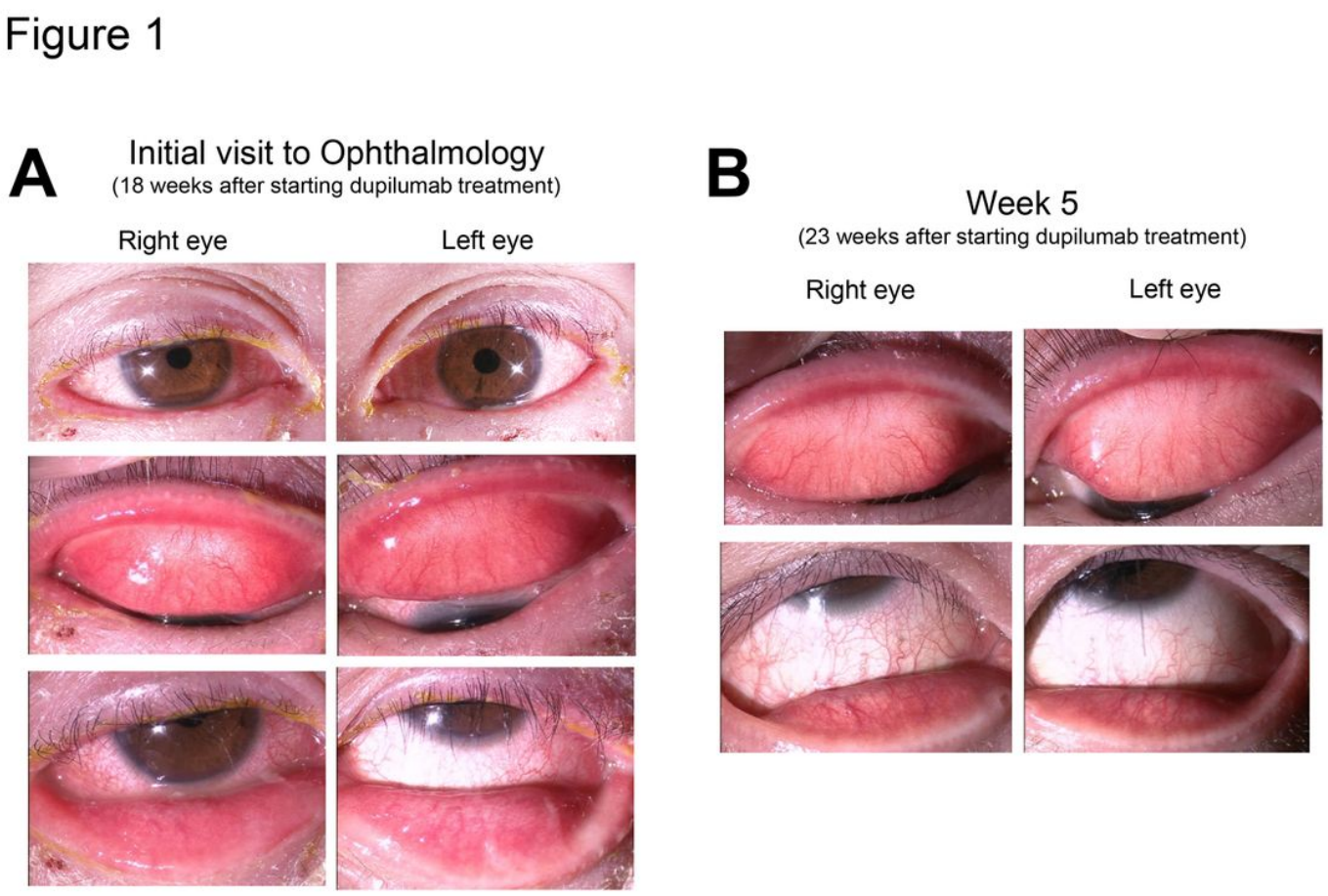

Figure 1

Photographs of blepharitis and conjunctivitis in case 1

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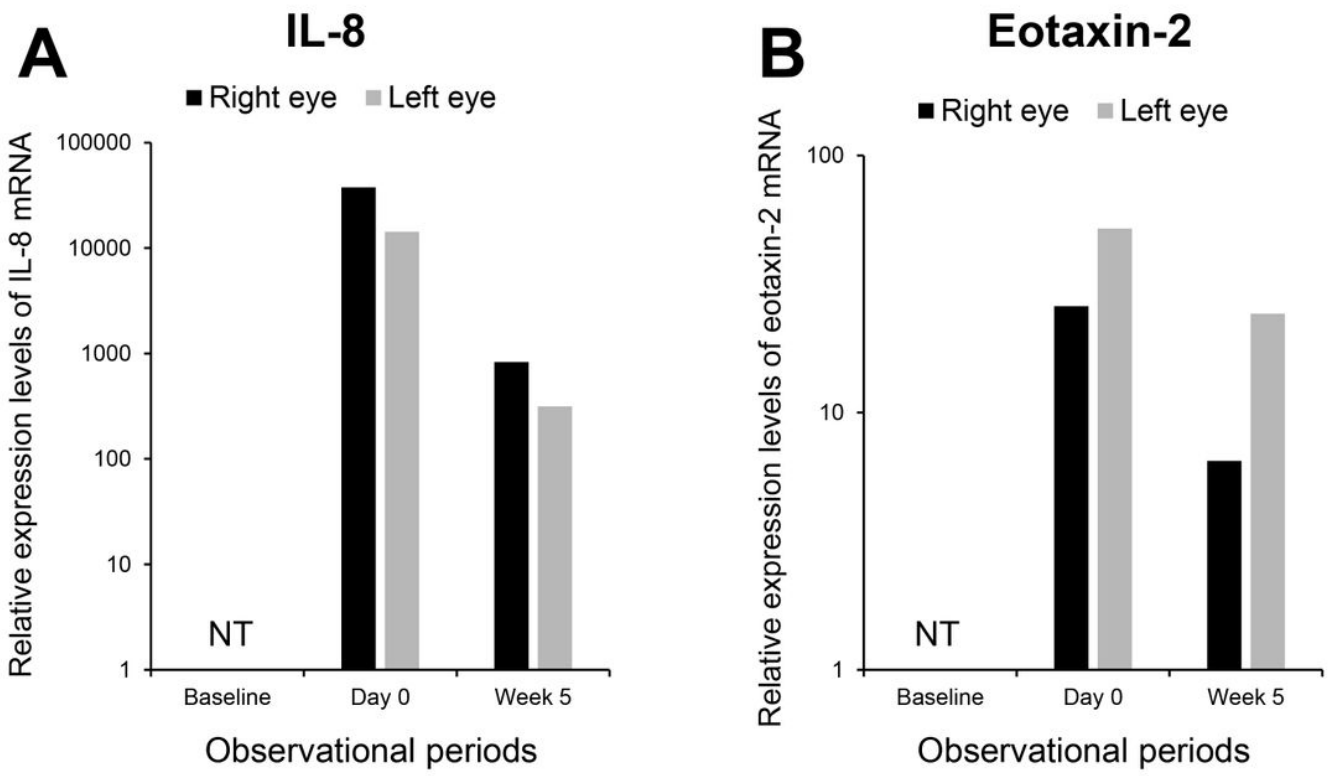
B. Five weeks after starting ophthalmologic treatment, clinical findings of blepharoconjunctivitis were improved.

Figure 2



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NT, not tested.

Figure 3

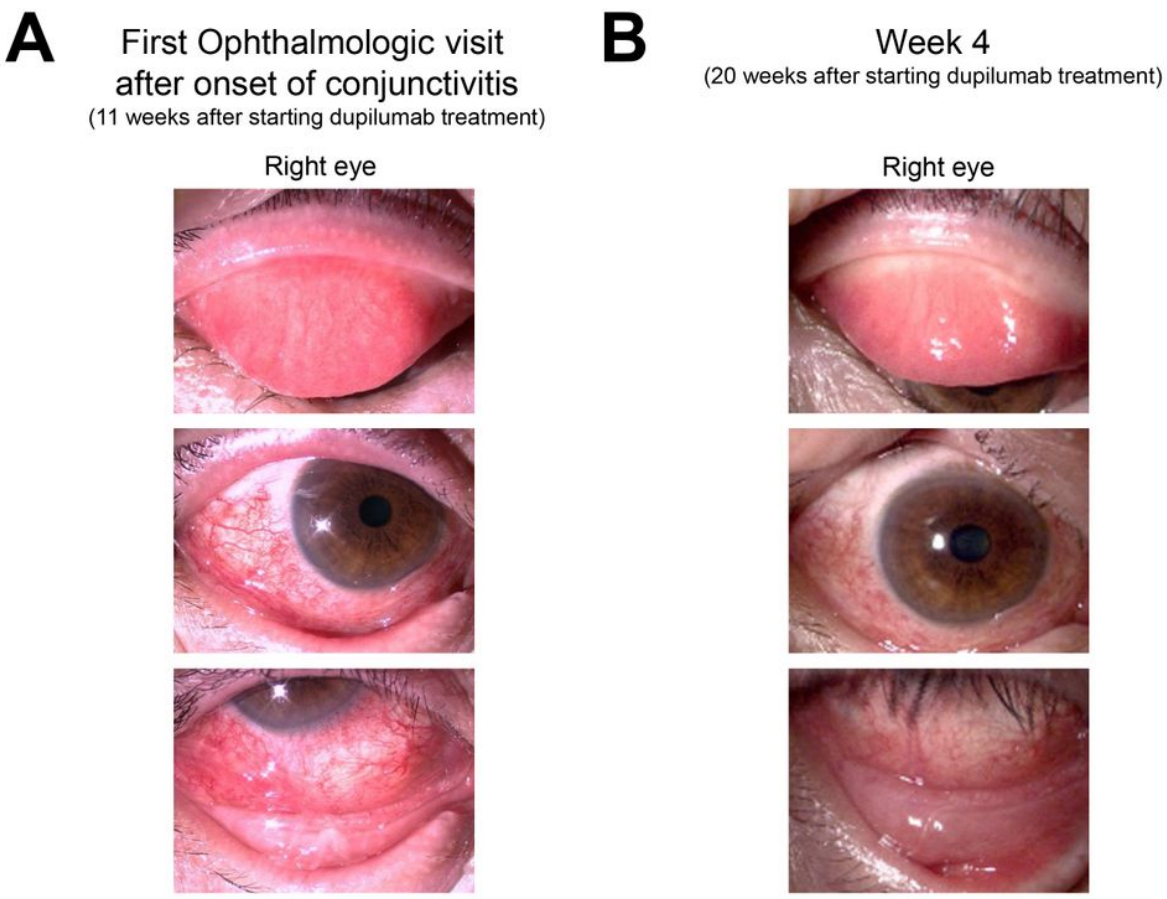

Figure 3

Photographs of blepharitis and conjunctivitis in case 2

A. At the first ophthalmology visit after the onset of conjunctivitis, conjunctivitis with a velvety appearance of palpebral conjunctivitis and severe bulbal hyperemia were observed.

B. Nine weeks after starting ophthalmologic treatment, the clinical findings of conjunctivitis mostly resolved, but mild hyperemia continued.

Figure 4

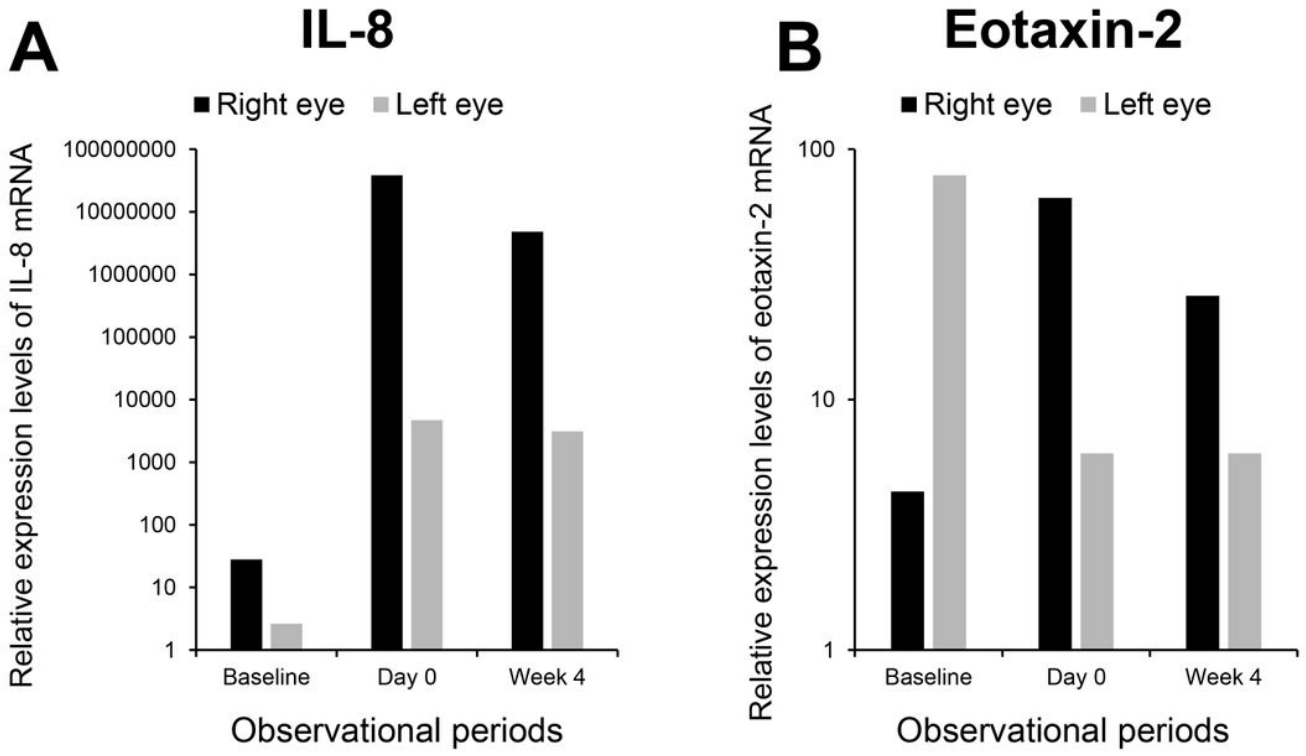

Figure 4

Results of ocular surface test for IL-8 and eotaxin-2 in case 2.

A. Relative expression of IL-8 mRNA on the ocular surface. The IL-8 mRNA levels peaked in both eyes at the onset of conjunctivitis (day 0) and decreased thereafter.

B. Relative expression levels of eotaxin-2 mRNA on the ocular surface. Eotaxin-2 mRNA levels were mildly elevated in (right eye) or virtually unchanged (left eye) with the onset of conjunctivitis.