cells. Moreover, PK-initiated, immunologically driven ocular surface inflammation may induce persistent epithelial defects and corneal melting, perforation, or both, ultimately resulting in blindness.[12] Allograft transplantation of healthy limbal tissue is useful for the reconstruction of the ocular surface. However, long-term outcomes are poor in eyes with SJS or TEN.[19] Groundbreaking surgical procedures have been developed over the past 12 years. We first reported the usefulness of cultivated corneal epithelial transplantation for SJS with persistent epithelial defects after the acute stage.[20–23] In another report, we clarified the efficacy of ex vivo expanded autologous oral mucosal epithelial cells to the ocular surface.[24] Cultivated oral mucosal epithelial transplantation and the 2-step surgical combination of cultivated oral mucosal epithelial transplantation and PK have provided the patients with SJS or TEN with a surgical pathway toward restoration of their visual function.[25–27] However, it is impossible for the ocular surface of those patients to be restored to its previously normal state.

Diagnosis of SJS or TEN at disease onset is complex, often confusing, and very difficult. Moreover, the use of steroids for treatment remains controversial.[10,28–30] Our recent reports and those of others indicated the influence of genetic endowment in SJS and TEN.[31–40] For instance, there are statistically significant differences in single nucleotide polymorphisms of toll-like receptor 3, interleukin (IL)-4R/IL-13, and Fas ligand in SJS and TEN; thus, genetic screening may help to deliver a more rapid diagnosis in the future. At present, however, the understanding of the typical clinical picture of SJS and TEN is still a vital aspect of early diagnosis and the initiation of treatment. Therefore, this study investigated the clinical manifestation at disease onset of SJS and TEN with ocular complications and evaluated the relationship between ophthalmic management at the acute stage and the visual outcomes.

Patients and Methods

From November 2005 through May 2008, extensive interviews were conducted with 94 patients (45 males and 49 females) with SJS or TEN with ocular complications seen at the SJS outpatient service at Kyoto Prefectural University Hospital. Of those patients, 88 cases were referral patients from the greater Japan area who had come to the SJS service at the acute stage (n = 14) or at the chronic stage (n = 74). Their ages ranged from 1 to 83 years (mean age±standard deviation, 41.6±18.5 years). At disease onset, the patients' ages ranged from 0 to 77 years (mean age±standard deviation, 26.2±18.8 years), and the duration of the illness ranged from 1 to 48 years (mean±standard deviation, 16.1±15.2 years). The questionnaires used in this study were structured as follows: (1) age of the patient at disease onset; (2) causative drugs; (3) the presence of prodromal symptoms; and (4) the episodes of high fever, conjunctivitis, skin eruptions, fingernail loss, and associated mucous membrane involvements. Medical records also were examined or the patients were asked directly regarding any ophthalmic management, especially the use of topical steroids, during the first week from disease onset. Then, the Mann–Whitney *U* test was used to analyze the correlation between the use of topical steroids and the visual outcomes. This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan.

The diagnosis of SJS or TEN at the acute stage was based on the acute onset of high fever, serious mucocutaneous illness with skin eruptions, involvement of at least 2 mucosal sites, and the pathologic findings of a skin biopsy that demonstrated necrotic changes of the dermis. The diagnosis of SJS or TEN at the chronic stage was based on ocular cicatricial findings such as symblepharon, severe dry eye, corneal neovascularization, opacification, and conjunctivalization, and a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites including the ocular surface. In the patients where disease onset occurred before age 10 years or in those who had lost consciousness at the acute stage because the illness, specific details were obtained by directly interviewing members of the immediate family.

Results

Of the 94 patients, drugs were the most commonly associated etiologic factor in 84 patients (89.4%). The causative drugs were cold remedies in 30 patients, antibiotics in 23 patients, nonsteroidal anti-inflammatory drugs in 19 patients, anticonvulsants in 5 patients, and others (anticancer agents, antirheumatic drugs, antimalarial, Chinese medicine, etc.).

Best-corrected visual acuity obtained at the chronic stage was 20/20 or better in 34 eyes (18.3%; Fig 1A), worse than 20/20 and up to and including 20/200 in 55 eyes (29.6%; Fig 1B), worse than 20/200 and up to and including 20/2000 in 53 eyes (28.5%; Fig 1C), and worse than 20/2000 in 44 eyes (23.7%; Fig 1D). Two eyes of 1 boy who was 1 year or age were excluded from the results because his visual acuity could not be assessed.

Characteristics of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis with Ocular Complications

Of the 94 patients, common coldlike symptoms (general malaise, fever, sore throat, etc.) preceded skin eruptions in 75 patients. Extremely high fever (more than 39° C) was reported by 86 patients, whereas 1 patient reported no fever and the remaining 7 patients could not remember the extent of the fever. Acute conjunctivitis and oral involvements (blisters, erosions, and bleeding of the mouth and lips) occurred in all patients who could recollect their symptoms in detail. Fingernail loss at the acute stage or deformation at present existed in all patients (Table 1; Fig 2). Other mucous membrane involvements included those of the pharynx, respiratory tract, or ear canal.

Forty-two patients reported episodes of acute conjunctivitis several hours to 4 days before the skin eruptions, and 21 patients reported that skin eruptions and conjunctivitis occurred simultaneously. Only 1 patient reported posteruption conjunctivitis (Table 2).

Topical Steroid Instillation and Visual Outcomes

Thirty-three patients (13 males and 20 females; mean age±standard deviation at disease onset, 31.5±18.6 years) began topical steroid treatment during the first week from disease onset, whereas 31 patients (14 males and 17 females; mean age±standard deviation, 27.9±19.5 years) received no topical steroid treatment or any other treatment for their eyes. The remaining 30 patients could not recall the details of ocular management during the first week from disease onset. Visual outcomes were significantly better in the group that received topical steroids at the acute stage compared with those of the no-treatment group ($P<0.00001$; Fig 3).

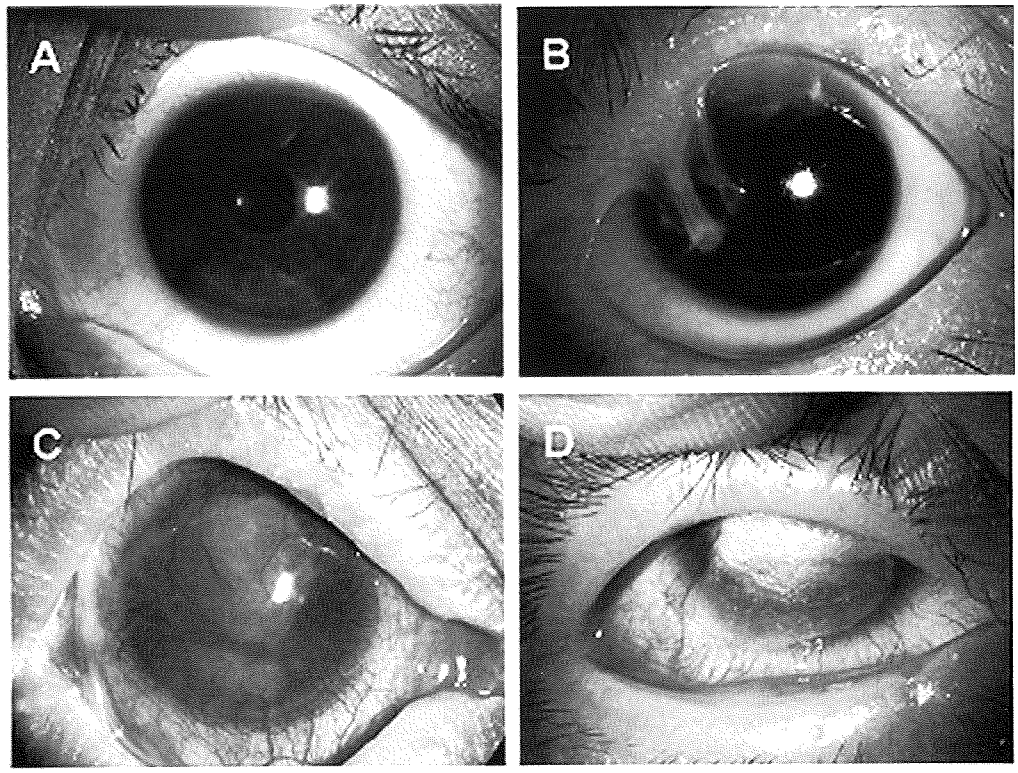

Figure 1. Photographs showing representative ocular manifestations at the chronic stage, with corresponding visual acuity. A, Clear cornea and best-corrected visual acuity of 20/20 or better: 34 eyes (18.3%). B, Moderate conjunctivalization and visual acuity worse than 20/20 and up to and including 20/200: 55 eyes (29.6%). C, Severe conjunctivalization and neovascularization and visual acuity worse than 20/200 and up to and including 20/2000: 53 eyes (28.5%). D, Keratinization, severe opacification, and visual acuity worse than 20/2000: 44 eyes (23.7%).

Diagnosis at the Acute Stage

Eleven patients were diagnosed with acute conjunctivitis by ophthalmologists before the development of systemic eruptions. An additional 12 patients were misdiagnosed as having measles (n = 4), chickenpox (n = 2), herpetic infection (n = 2), rubella (n = 1), or other diseases by physicians in other fields.

Among 94 patients, only 37 patients were diagnosed as having SJS or TEN at disease onset. Seven patients were diagnosed properly at several weeks (range, 2–8 weeks) after the onset, and surprisingly, 6 patients obtained the diagnosis at 2 to 45 years after the onset. For the remaining patients, when they received a proper diagnosis could not be ascertained.

Table 1. Symptoms and Mucosal Involvements of the 94 Patients at the Acute Stage

Symptoms	Experienced	Did Not Experience	Unknown
Prodromal common cold-like symptoms	75	17	2
Extremely high fever (>39° C)	86	1	7
Ocular involvement	94	0	0
Oral involvement	82	0	12
Genital involvement	46	18	30
Fingernail loss or deformation	94	0	0

Discussion

Stevens-Johnson syndrome and TEN are rare but potentially fatal skin disorders. Ocular involvement is common and often results in long-term complications such as serious visual impairment with ocular discomforts.[13,28] Although much has been learned over the past 50 years about the management of SJS and TEN, the following 3 important problems still remain: (1) the difficulty of obtaining a prompt and accurate diagnosis of SJS or TEN at disease onset, (2) ocular involvement often is overlooked easily because of the serious general symptoms and high lethality of these 2 diseases, and (3) a universally accepted treatment regimen for SJS and TEN has yet to be adopted and treatment with corticosteroids remains controversial.[10,28–30] There is also no standardized ophthalmologic treatment for the prevention of ocular complications.

In this study, 12 patients were misdiagnosed as having chickenpox, measles, herpetic infection, or other diseases. For early diagnosis, the clinical pictures of SJS and TEN need to be well understood, and to that end, the results of this study provided new and important data. Common cold-like symptoms (general malaise, slight fever, sore throat, etc.) preceded skin eruptions in 82% of the cases, and in all but 1 patient, the disease was accompanied by very high fever (more than 39° C) at the onset. It should be emphasized that acute conjunctivitis occurred before or simulta-

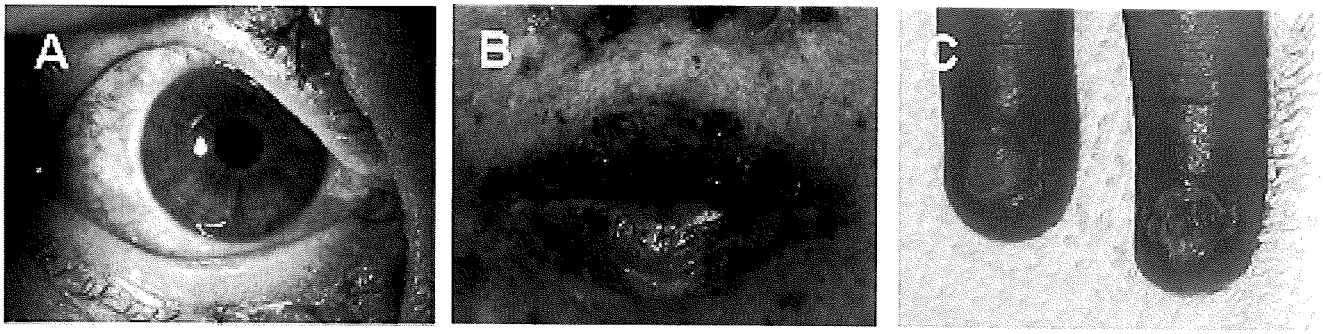

Figure 2. Representative photographs showing Stevens-Johnson syndrome/toxic epidermal necrolysis-associated ocular and oral involvement and fingernail loss at the acute stage. A, Conjunctivitis, which was accompanied by extensive loss of bulbar conjunctival epithelium. B, Swollen and crusted lips. C, Fingernail loss and deformation with paronychia.

neously with skin eruptions and that the involvement of oral mucosa was observed in 100% of the cases who could remember the details. Fingernail loss at the acute stage, deformation at the time of the writing of this report, or both also occurred in all of the patients, suggesting that paronychia occurred in all patients at the acute phase.

Visual outcomes were significantly better in the patients who received treatment with topical steroids during the first week from disease onset compared with those of the patients who received no topical steroid treatment. However, those outcomes may be because of the presumed fact that patients who fail to receive treatment with topical steroids are highly likely not going to receive systemic steroids as well. Thus, treatment with topical steroids, systemic steroids, or both at the early stage of the disease helps to decrease the incidence of chronic ocular complications. At the onset of the diseases, both necrotic changes of the skin and the destruction of the ocular surface progress rapidly. Prompt use of topical steroids, and presumably systemic steroids, from disease onset may prove to be important for preventing the loss of corneal epithelial stem cells. Unfortunately, a detailed history concerning the systemic therapy during the acute stage could not be obtained in most instances. Additional studies are needed to confirm the safety and efficacy of those medications.

Of the 94 patients, the mean duration of the illness was 16.1 years, and more than 50% of the eyes manifested visual acuity worse than 20/200. Considering the fact that patients with SJS or TEN experience ocular complications for an extended period, it is vital that strict attention be paid to any ocular involvement. When dermatologists, physicians, and healthcare professionals suspect SJS or TEN, prompt referral to an ophthalmologist is vital for the prevention of permanent loss of vision. Ophthalmologists have to find distinctive appearances such as pseudomembrane formation and corneal or conjunctival epithelial defects, or both.

Table 2. Order of Conjunctivitis and Skin Eruptions of the 94 Patients at Disease Onset

Conjunctivitis	Period Preceding Eruption	No. of Patients
Occurred before skin eruption	4 days	1
	3 days	3
	2 days	11
	1 day	12
	Several hours	9
	Unknown	6
		Total = 42
Occurred simultaneously		21
Occurred later		1
Unknown		30
Total		94

In the first report by Stevens and Johnson, 2 boys reported eye pain before skin eruptions and manifested a purulent conjunctivitis. Visual prognosis was total blindness in one case and severe corneal scarring in the other case. Both cases had the typical clinical picture clarified in the present study.[1] If their eyes had been treated with topical steroids from disease onset, the visual outcomes might have been different.

To date, the pathophysiologic mechanisms underlying the onset of SJS and TEN have yet to be fully elucidated. The rarity of these diseases has led us to speculate that patients with SJS or TEN genetically are susceptible to specific environmental precipitants. A report from the United States showed an increase of human leukocyte antigen (HLA)-B12 (HLA-Bw44) antigen in white patients with SJS with ocular involvement.[31] Analyses of TEN patients in France also disclosed an association with HLA-B12 (HLA-Bw44).[32] In Han Chinese, there was a very strong association between carbamazepine-induced SJS and the HLA-B*1502 allele.[33] The authors also reported that in Japanese persons, HLA-A*0206 was strongly associated with SJS and TEN with ocular surface complications.[34,38] These findings suggest that SJS and TEN are associated with a complex genetic inheritance background.

The prodromal symptoms occurred in 82% of the cases in this study. Given the association between the onset of SJS and TEN and infections and the opportunistic infection of ocular surfaces by bacteria such as methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis*,[41] it is highly possible that there is an association between SJS and TEN and a disordered innate immune response. Recently, the association of the polymorphisms in the toll-like receptor 3 gene with SJS and TEN in the Japanese population were reported.[36] Also, an association between SJS and TEN and the IL-4R gene polymorphism and combined IL-13/IL-4R signaling pathway gene polymorphism was reported.[35,39]

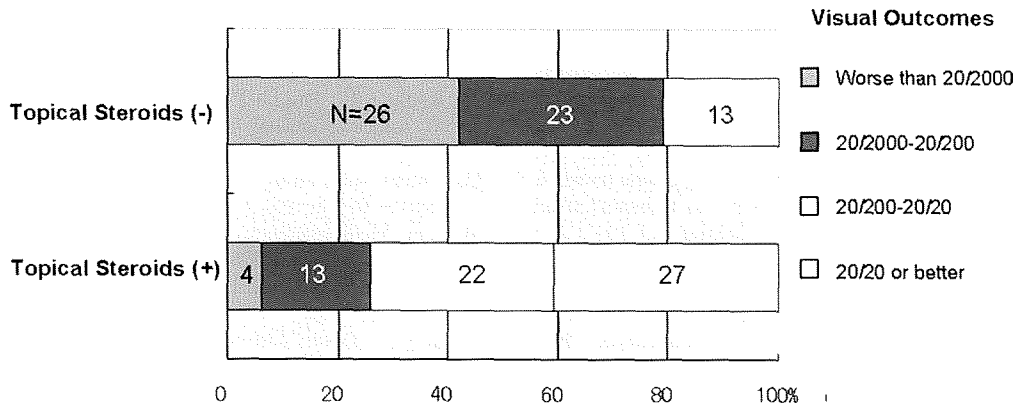

Figure 3. Graph showing the relationship between topical steroid use during the first week from disease onset and visual outcomes. Sixty-six eyes of 33 patients began topical steroid treatment during the first week from disease onset, whereas 62 eyes of 31 patients received no topical steroid treatment or any other treatment. Visual outcomes were significantly better in the group receiving topical steroids at the acute stage compared with those of the no-treatment group ($P<0.00001$).

Thus, both innate immunity and host-defense mechanisms may play a critical role in the development of SJS and TEN.

In conclusion, ocular involvement at disease onset is a helpful symptom for the diagnosis of SJS and TEN. Acute conjunctivitis before or occurring simultaneously with skin eruptions accompanied by very high fever and blisters on the mouth greatly implies the initial signs of SJS and TEN, and prodromal symptoms and genital involvements support that diagnosis. Initiating treatment with topical steroids from the onset seems to be important for the improvement of visual prognosis. A prompt and accurate diagnosis as assisted by the clinical manifestation offers a breakthrough against the historically poor visual outcomes associated with patients with SJS or TEN.

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Footnotes and Financial Disclosures

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Successful Treatment of Stevens-Johnson Syndrome with Steroid Pulse Therapy at Disease Onset

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• PURPOSE: To evaluate the visual prognosis of patients with Stevens-Johnson syndrome (SJS) and its severe variant, toxic epidermal necrolysis (TEN), followed by general and topical high-dose corticosteroids administration from disease onset.

• DESIGN: Prospective, observational case series.

• METHODS: Between May 1, 2003 and June 30, 2005, we enrolled 5 patients with SJS or TEN with ocular complications at the acute stage. Intravenous pulse therapy with methylprednisolone (steroid pulse therapy; 500 or 1000 mg/day for 3 to 4 days) was initiated within 4 days from disease onset. Topically, 0.1% betamethasone was applied over 5 times daily for at least 2 weeks. Visual acuity (VA) and slit-lamp microscopic appearance 1 year from disease onset were evaluated.

• RESULTS: At the first examination, corneal or conjunctival epithelial defects and pseudomembranous conjunctivitis were present in all cases. Skin eruptions dramatically improved after steroid pulse therapy. Although ocular inflammation increased for several days, pseudomembranes disappeared and corneal and conjunctival epithelium regenerated within 6 weeks. At the chronic stage, all eyes had clear corneas with the palisades of Vogt (POV), implying the presence of corneal epithelial stem cells. Best-corrected VA was 20/20 or better in all eyes. Five eyes showed superficial punctate keratopathy. No eye had cicatricial changes except for 1 with slight fornix shortening. No significant adverse effects of steroid occurred during all clinical courses.

• CONCLUSIONS: Steroid pulse therapy at disease onset is of great therapeutic importance in preventing ocular complications. Topical betamethasone also shows great promise for preventing corneal epithelial stem cell loss in the limbal region and cicatricial changes. (Am J Ophthalmol 2009;147:1004–1011. © 2009 by Elsevier Inc. All rights reserved.)

See accompanying Editorial on page 949.

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STEVENS-JOHNSON SYNDROME (SJS), FIRST REPORTED in 1922, is an acute inflammatory disease that predominantly affects the skin and mucosal membranes, including the ocular surface.[1] In 1956, Lyell described a clinical condition characterized by extensive epidermal loss, termed *toxic epidermal necrolysis* (TEN).[2] Recent reports suggest that SJS and TEN are the same disorder, but of different severities.[3–5] In the acute stage, these diseases predispose patients to life-threatening complications such as sepsis, respiratory dysfunction, and multiorgan failure. Mucosal sites including the ocular surface and oral membrane commonly are involved at the onset of acute fever and skin eruptions. Although skin usually heals without dysfunction, severe corneal opacity and dry eye often persist during the chronic stage. Patients with SJS or TEN require life-long management for ocular discomfort and morbidity.[6–9] Recently, it was reported that amniotic membrane transplantation (AMT) onto the ocular surface is effective for reducing the destructive inflammation in acute SJS or TEN and for preventing cicatricial change.[10–12] However, safe and effective medical treatment for the prevention of ocular complications has yet to be established.

Although the pathogeneses of SJS and TEN has not been elucidated fully, it has been indicated that soluble FasL-mediated apoptosis plays a crucial role in the pathogeneses of SJS and TEN.[13] Drug-specific cytotoxic CD8+ T lymphocytes were detected in blister fluids of SJS and TEN patients and in cytotoxic lymphocyte cytolytic pathways, including major histocompatibility complex class I.[14] It also has been reported that tumor necrosis factor and interferon-γ also are involved in the mechanisms of epidermal necrosis.[15] Therefore, it is highly possible that medication at the acute stage to downregulate such immunologic reactions is useful for the treatment of SJS and TEN.

The use of systemic corticosteroids for the care of patients with acute SJS and TEN is controversial.[16,17] Although the beneficial effects of corticosteroid therapy during the acute stage has been reported,[18–20] high mortality rates in patients receiving corticosteroids has been shown.[21,22] The timing, dose, formulation, and route of administration of the steroid differ in these reports. At disease onset, skin involvement and ocular involvement progress rapidly, and facial manifestation and general condition became worse from morning to evening. Considering the pathogenesis described above and the rapid progression of SJS and TEN at disease onset, we hypoth-

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esized that the timing and dose of the administered steroid are both key to obtaining beneficial effects.

In patients with SJS- or TEN-induced chronic ocular complications, the total loss of the palisades of Vogt (POV) commonly is observed.[23] POV in the limbal area implies the presence of corneal epithelial stem cells.[24] At the acute stage, corneal epithelial defect or corneal ulceration occur in more than 50% of the patients with SJS or TEN.[25] In cases with limbal stem cell loss, conjunctivalization and neovascularization of the cornea progress, leading to severe visual impairment or blindness.[6-9] Loss of the POV occurs during the acute stage of SJS and TEN and can be accompanied by severe inflammation. The administration of high-dose general and topical corticosteroids from disease onset may downregulate the immunologic reactions described above and may prevent the loss of corneal epithelial stem cells.

The aim of this study was to evaluate the ophthalmic efficacy of high-dose corticosteroid therapy at the acute stage of SJS or TEN. All patients in this study were administered high-dose systemic methylprednisolone (steroid pulse therapy) and topical betamethasone for SJS or TEN with ocular involvement from the onset of the disease. Side effects of the steroids were monitored carefully over the duration of this study, and a great amount of attention was paid to systemic and ophthalmic infections. We then evaluated visual acuity (VA) and the slit-lamp microscopic appearance in these patients at the chronic stage.

METHODS

BETWEEN MAY 1, 2003 AND JUNE 30, 2005, WE ENROLLED 5 consecutive patients (2 males and 3 females, 23 to 49 years of age at disease onset; mean age, 32.8 years) referred to us during the first 4 days from the onset of SJS or TEN accompanied by ocular complications (ocular surface epithelial defects, pseudomembranous formation, or both). The diagnosis of SJS or TEN was confirmed by dermatologists based on clinical and histopathologic classification.[26,27] Prior informed consent to participate in the study was obtained in written form from all patients, their families, or both.

We initiated therapy with intravenous high-dose methylprednisolone and intensive topical betamethasone immediately after the dermatologic and ophthalmologic diagnosis. For initial treatment, the protocol used in this study was as follows: intravenous methylprednisolone at a dosage of 500 to 1000 mg/day was used for 3 to 4 days (steroid pulse therapy) and 0.1% betamethasone was applied topically more than 5 times daily for at least 2 weeks. The topical antimicrobial agent was applied prophylactically.

Signs of systemic and ophthalmic infection were monitored by the culture of blood, conjunctival swab, and the swab of other mucous membranes. The body temperature and the patient's symptoms and biochemical parameters also were monitored carefully.

Patient-related ocular findings and the complications during the acute stage were recorded fully until the remission of the ocular surface inflammation. As for ocular complications, corneal complications (superficial punctate keratopathy [SPK], epithelial defect, loss of the POV, conjunctivalization, neovascularization, opacification, and keratinization), conjunctival complications (hyperemia and symblepharon formation), and eyelid complications (trichiasis, mucocutaneous junction involvement, meibomian gland involvement, and punctal damage) were recorded according to a new grading system for SJS that we previously reported.[23] Tear secretion was assessed by the Schirmer 1 test, and meibomian gland morphologic features were evaluated using meibography.[28,29] VA and ocular findings at the chronic stage were evaluated after 1 year from the onset of the disease.

RESULTS

• OCULAR FINDINGS AND THERAPY DURING THE ACUTE STAGE: Five patients were referred to us within 4 days (0 to 4 days; mean, 1.2 days) from the onset (the initiation of skin eruptions accompanying mucocutaneous illness) of the disease (Table). All patients had rapidly progressing skin eruptions, mucous membrane erosions, and a very high fever (more than 39 C) at presentation. Those symptoms were preceded by common cold-like symptoms (general malaise, fever, sore throat, or a combination thereof) in 4 patients. The causative drugs were cold remedies (Cases 1 and 5), antibiotics (Cases 3 and 5), and nonsteroidal anti-inflammatory drugs (Cases 3, 4, and 5). In 1 patient, high fever and erythematous macules developed after vaccination for measles (Case 2). All 10 eyes had pseudomembranous conjunctivitis. Corneal or conjunctival epithelial defects were present in all cases. Corneal epithelial defects existed in 5 eyes, and severe SPK was present in the other 5 eyes. Conjunctival epithelial defects were observed in 6 eyes (Figures 1 and 2). Skin biopsy specimens of the erythematous macules from all patients showed necrotic keratinocytes and liquefaction degeneration that were consistent with the diagnosis of SJS or TEN (Figure 3).

In all patients, steroid pulse therapy was initiated immediately after confirming ocular involvement, except 1 case (Case 5) in which steroid pulse therapy already had been initiated previously by a dermatologist. Thereafter, systemic steroids were changed to prednisolone or betamethasone (Table). Topically, 0.1% betamethasone (0.1% betamethasone solution, 0.1% betamethasone eye ointment, or both; 5 to 8 times daily) was used from the day we confirmed ocular involvement. An ophthalmic fluoroquinolone solution (0.3% gatifloxacin or 0.3% ofloxacin; 4 times daily) was used for the prevention of ocular infec-

TABLE. Dosage and Duration of Systemic Corticosteroid Administration during the Acute Stage of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

				Steroid Pulse Therapy (Methylprednisolone)			Steroid Administration after Pulse Therapy (Prednisolone Equivalent)		
Case	Diagnosis	Age (yrs)	Gender	Elapsed Time from Onset to Initiation of Therapy (days)	Steroid Dose (mg/day)	Duration (days)	Initial Dose (mg/day)	Total Duration (days)	Total Amount (mg)
1	SJS	23	M	1	500	3	40	85	1045
2	SJS	27	F	0	1000	3	40	35	510
3	SJS	31	F	4	500	3	60	20	425
4	SJS	34	F	1	1000	3	40	72	570
5	TEN	49	M	0	500	4	60	9	420
Mean		32.8		1.2				44	594

F = female; M = male; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis; yrs = years.

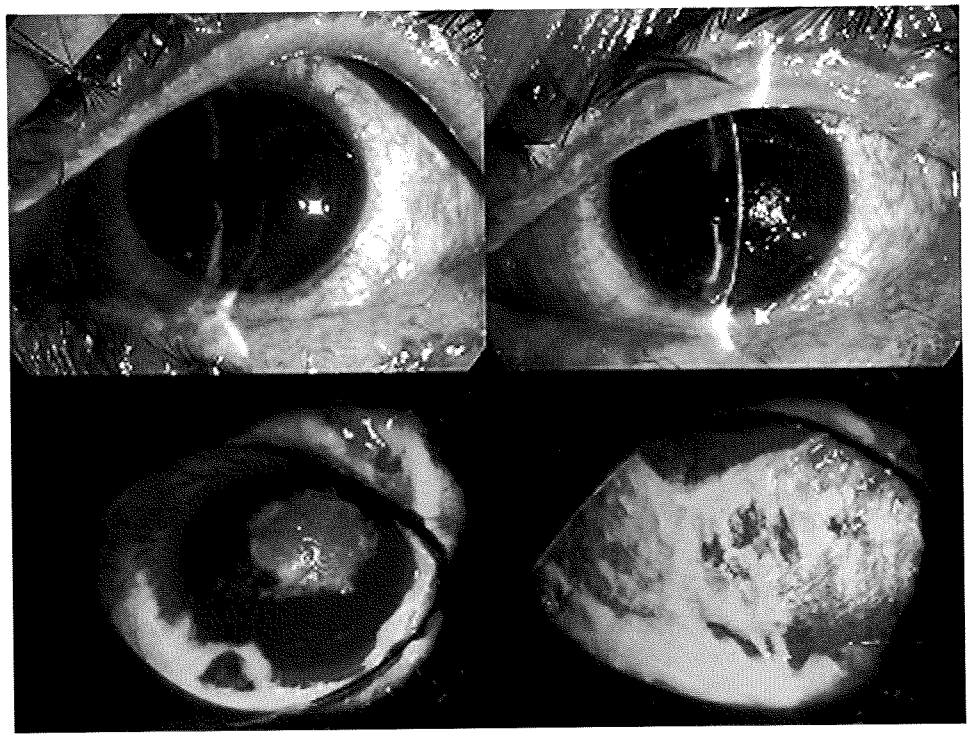

FIGURE 1. Images demonstrated Stevens-Johnson syndrome (SJS) at the acute stage (Case 1). (Top left) Two days after disease onset, pseudomembranous conjunctivitis with conjunctival epithelial defect was present around the limbus. The cornea was clear without defect and superficial punctate keratopathy. (Top right) At the most inflamed phase, 9 days after disease onset and 8 days after the start of steroid pulse therapy, the ocular surface was most inflamed with increased eye discharge, and the pseudomembrane and cilia fell out partially in the lower eyelid. (Bottom left) Corneal epithelial defect. (Bottom right) Conjunctival epithelial defect extending to almost the entire bulbar and palpebral conjunctiva.

tions. Prophylactic systemic antibiotics were not used, because all 5 cases were associated with drug reactions.

Skin eruptions dramatically improved after initiation of the steroid pulse therapy (Figure 4). Despite intensive use of systemic and topical corticosteroids, pseudomembranous formation increased and epithelial defects enlarged during the first several days, peaking at 1 to 9 days (mean, 4.0 days) from their onset. Thereafter, corneal epithelial defects improved day by day and disappeared within 2 to 13 days (mean, 5.2 days). Conjunctival epithelium regenerated completely within 1 to 38 days (mean, 13.0 days).

The administration of systemic steroids was tapered off gradually according to the patient's general and ophthalmic conditions. Whereas cutaneous involvement was quickly eliminated after initiation of steroid pulse therapy, ocular surface inflammation tended to persist longer than cutaneous inflammation. The total amount of steroids was 420 to 1045 mg (changed to prednisolone) during 9 to 85

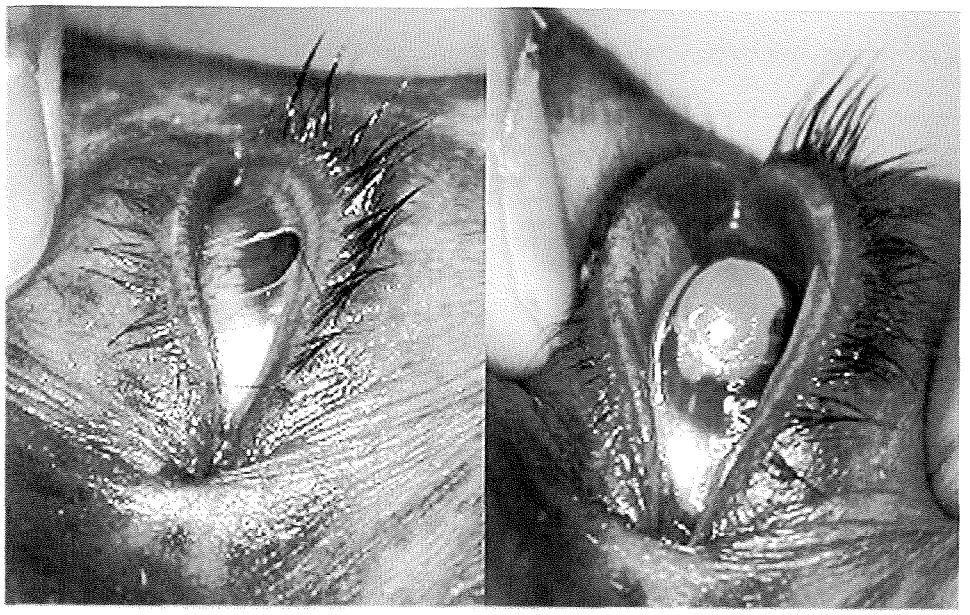

FIGURE 2. Images showing toxic epidermal necrolysis at the acute stage (Case 5). Because the general condition was still critical, the patient was examined on his bed. (Left) Pseudomembrane was present between the upper and lower eyelids. (Right) After removal of the pseudomembrane, corneal epithelial defect was observed.

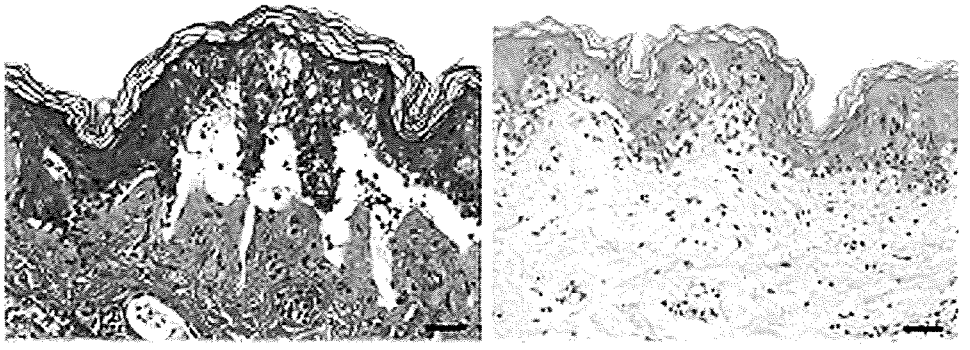

FIGURE 3. Photomicrographs showing sections from a skin lesion of SJS or toxic epidermal necrolysis at the acute stage. (Left) Case 1 with SJS (Right) Case 5 with toxic epidermal necrolysis. These sections show liquefaction degeneration producing a subepidermal cleft. The epidermis contains numerous necrotic keratinocytes with vacuolated cytoplasm or pyknotic nucleus (hematoxylin and eosin, bars = 100 mm).

days from disease onset (Table). One patient with TEN received plasmapheresis[30,31] for 3 days after steroid pulse therapy. Topical 0.1% betamethasone was used for a total of 40 to 165 days (mean, 91.4 days), then switched to 0.1% fluorometholone.

We observed no significant adverse effects from steroid pulse therapy, such as sepsis, pneumonia, or other infections. No cardiac arrhythmia or kidney or liver dysfunction occurred. We continued the culture of the conjunctival swabs during the use of topical or systemic steroids, or both. Methicillin-resistant *Staphylococcus aureus* was detected from the culture of the conjunctival swab in 2 eyes of 1 case at 1.5 months from disease onset, and coagulase-negative *Staphylococci* was observed in 2 eyes of another case at 10 days from disease onset. However, both cases showed no infectious ocular manifestations.

• VISUAL OUTCOMES AND OCULAR FINDINGS AT THE CHRONIC STAGE: In all eyes, best-corrected VA at 1 year from disease onset was 20/20 or better. No eyes had the appearance of an epithelial defect, the loss of the POV, conjunctivalization, neovascularization, opacification, or keratinization. As for corneal complications, only mild SPK was present in 5 eyes. As for conjunctival complications, fornix shortening with mild symblepharon was present only in 1 eye (Case 4). In contrast, all eyes manifested mild lid complications and mild irregularity of the mucocutaneous junction (Figure 5). All patients ex-

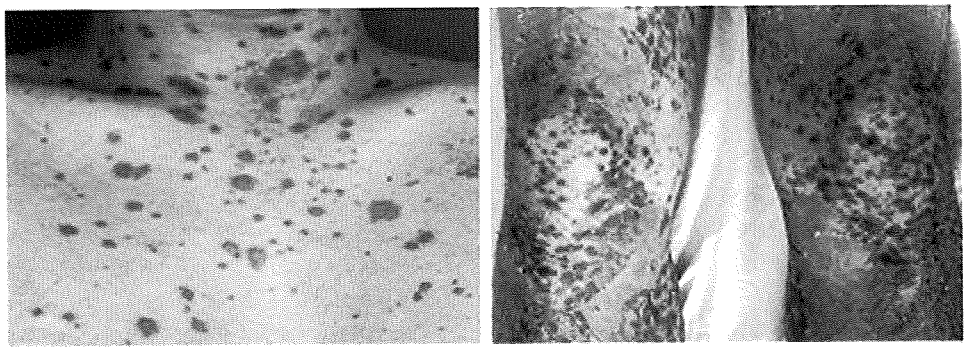

FIGURE 4. Photographs showing skin eruptions of SJS or toxic epidermal necrolysis after steroid pulse therapy. (Left) Case 1 with SJS. (Right) Case 5 with toxic epidermal necrolysis.

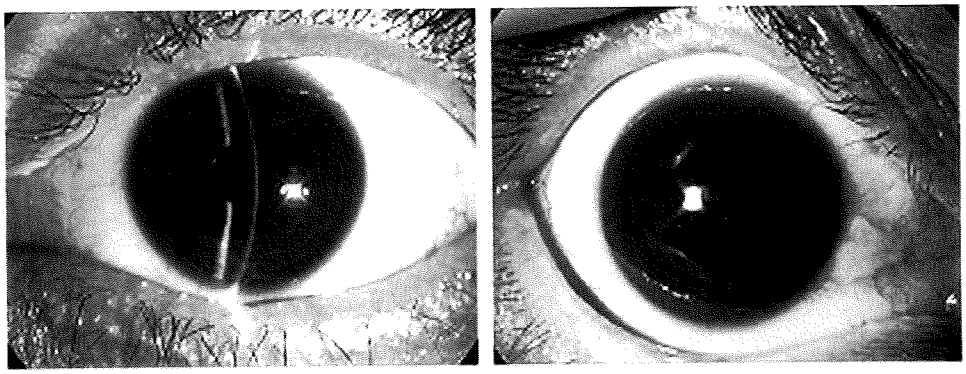

FIGURE 5. Photographs showing the ocular appearances of SJS or toxic epidermal necrolysis at the chronic stage. (Left) Case 1 with SJS. (Right) Case 5 with toxic epidermal necrolysis. The corneas were clear and the ocular surfaces were not inflamed 1 year after disease onset. Mucocutaneous junction involvements were mild.

perienced slight discomfort from irritation to their ocular surface, thus requiring the instillation of artificial tears. The Schirmer 1 test measured less than 5 mm in 4 eyes of 3 cases and 5 mm or more in the other eyes. There was no punctal damage in all eyes. Meibomian gland morphologic features were normal in 8 eyes and mild to moderately dropout in 2 eyes. No increase of intraocular pressure and no infectious keratitis occurred during all clinical courses.

• CASE 1: A healthy 23-year-old man (Case 1) presented to our hospital on October 14, 2004. He had erythematous skin eruptions on the trunk and extremities after taking cold remedies. The body temperature increased to more than 39 C and the erythematous macules increased rapidly and became blisters. Extensive hemorrhagic erosion on the lips and oral mucosa were also observed when he visited our hospital. He was aware of bilateral red eyes just before the skin eruptions. Eye discharge appeared with skin rashes and markedly increased as the skin and oral site worsened. At first examination, both eyelids were edematous, and pseudomembranous conjunctivitis was noted. The tarsal and bulbar conjunctivae were affected severely, and extensive epithelial defect was observed in both eyes. Lid margins also were ulcerated with the partial loss of cilia (Figure 1). A skin biopsy was performed and histopathologic findings were compatible with the clinical diagnosis of SJS (Figure 3).

Immediately after the diagnosis, steroid pulse therapy (methylprednisolone; 500 mg/day for 3 days) was initiated. Topically, betamethasone was instilled 8 times daily (eye drop and ointment each administered 4 times daily). The improvement was dramatic. First, the development of new lesions stopped after the initiation of steroid pulse therapy. Thereafter, skin eruptions decreased and systemic conditions improved day by day (Figure 4). However, ocular inflammation increased with pseudomembranous formation, bilateral epithelial defects in the center of the cornea, and large conjunctival epithelial defects extending to nearly the entire bulbar and palpebral conjunctiva. After the peak of ocular surface inflammation at 9 days from disease onset, corneal and conjunctival epithelium began to regenerate.

Steroid pulse therapy was switched to intravenous betamethasone at a dosage of 4 mg/day for 5 days and then gradually tapered off. The total amount of systemic steroid was 1045 mg of a prednisolone equivalent, administered for a total of 85 days. Topically, betamethasone was initially administered for 31 days, with a total duration of 165 days. The pseudomembrane was removed daily and

symblepharon was separated with a glass rod for 15 days. Although skin eruptions diminished without recurrence, pseudomembranous conjunctivitis recurred after the reduction of systemic or topical steroids at 2 to 4 weeks from disease onset. Both systemic and topical steroids were tapered off carefully according to the ocular surface appearance. Corneal epithelial defects healed within 3 days from their appearance. Large conjunctival epithelial defects needed 21 and 38 days for epithelization, respectfully. Ocular inflammation gradually subsided over a 5-month period.

At the chronic stage, 1 year from disease onset, VA was 20/20 in both eyes. Both corneas were clear with POV and there existed no symblepharon, trichiasis, conjunctivalization, or neovascularization. Only the mucocutaneous junction showed slight cicatricial changes with mild irregularity (Figure 5). The Schirmer 1 test showed 1 mm in the right eye and 2 mm in the left eye. Meibomian gland morphologic features were normal in both eyes. The patient used topical artificial tears, and mild SPKs existed. He reported either no or slight discomfort on the ocular surface.

DISCUSSION

AT THE BEGINNING OF THIS STUDY, WE HYPOTHESIZED that it is important to start steroid pulse therapy at the acute stage as soon as possible. Because the destruction of the ocular surface epithelium, especially corneal epithelial stem cells, at the acute stage progresses rapidly, the initiation time of corticosteroid therapy may be the key to obtaining a good prognosis. Based on this hypothesis, we prospectively used high-dose methylprednisolone and intensive topical betamethasone immediately after the dermatologic and ophthalmologic diagnosis in cases with ocular involvement.

Five cases of SJS, including 1 case of TEN, were enrolled in this study. We initiated steroid pulse therapy with methylprednisolone 0 to 4 days (mean, 1.2 days) from disease onset. Simultaneously, topical betamethasone treatment was initiated immediately. One year after disease onset, VA was 20/20 or better in all 10 eyes.

It is noteworthy that the POV were maintained completely in all eyes in this study, suggesting the survival of corneal epithelial stem cells. Although it is uncertain whether stem cell loss is the primary or secondary damage of SJS and TEN, it is probable that steroid pulse therapy at the onset of the disease protected corneal epithelial stem cells from depletion. Intriguingly, antioxidative agents reportedly restore the reconstitutive capacity of hematopoietic stem cells.[32] Corneal epithelial stem cells may be protected via the reduction of oxidative stress by high-dose corticosteroid instillation.

Previously, we reported that the most severely affected ocular components in SJS and TEN at the chronic phase were loss of the POV (82.6%) and meibomian gland involvement (73.9%).[23] In the cases reported in this study, there existed no cicatricial changes on the cornea, and only 1 eye showed fornix shortening. It is highly possible that intensive steroid therapy also prevented the destruction of the meibomian gland, thus resulting in much fewer lid-related complications. In 1 case (Case 1), pseudomembranous conjunctivitis recurred after the reduction of systemic or topical steroids. A careful reduction of general and topical steroids is necessary to prevent cicatricial changes of the ocular surface. Both the systemic and topical application of steroids is needed to suppress the ocular surface inflammation effectively, which sometimes persists for a longer period than the cutaneous inflammation.

Although this study was not a randomized trial, we also compared the patients with SJS and TEN at the chronic stage during the period of this research. Six patients (3 males and 3 females; 6 to 67 years of age at disease onset; mean age, 32.7 years) without systemic and topical steroids at the acute stage showed severe cicatricial changes of the ocular surface. Their VA was between hand movements and 40/200. Additional studies are needed to compare the systemic and ophthalmologic prognosis of SJS and TEN patients with or without the early administration of steroids.

Several reports have suggested the advantage of using cryopreserved AMT for the treatment of acute SJS and TEN.[10–12] The similarity between these reports and ours is the intervention of the treatment at the acute stage. It is noted that both treatments demonstrated the beneficial effects of reducing ocular surface inflammation at the acute stage, as well as positive results in preventing cicatricial changes at the chronic stage. It seems probable that early intervention limited the cicatricial changes later. Although the intensive steroid therapy in this study was started within 4 days from disease onset, the timing of AMT in the previous reports was later than the initiation of treatment shown in our study.[12] In addition, the use of systemic and topical steroids was not described in the previous reports. For these reasons, we are unable to compare the effect of AMT and steroid pulse therapy on the ocular complications in SJS and TEN. Further studies are needed to compare and elucidate the effects, indication, and complications associated with treatment by surgery vs medical intervention.

It should be emphasized that in this study, steroid pulse therapy was initiated within 4 days from disease onset. If extensive mucocutaneous damage already has progressed, the risks of general and local infections increase. Although all 5 cases in this study were associated with drug reactions, not all cases of SJS and TEN are caused by drugs. A fraction of cases are caused by viral (such as herpes simplex virus) or mycoplasma infection. It is important to confirm that there are no signs of infectious activity before the administration of steroid pulse therapy by monitoring vital signs and by detecting potential pathogens by serum

antibody titer, blood culture, and the polymerase chain reaction method. Intensive management by a medical team consisting of at least 1 dermatologist, 1 ophthalmologist, 1 physician, and 1 infection control doctor is needed to obtain the best results.

Our recent reports, and those of others, have indicated the participation of genetic endowment in SJS and TEN.[33–41] For instance, there are statistically significant differences in single nucleotide polymorphisms of toll-like receptor 3 interleukin 4R/interleukin 13, and FasL in SJS and TEN; thus, genetic screening may help to deliver a more rapid diagnosis or prevention of SJS and TEN in the future. At present, however, prompt diagnosis and early treatment with high-dose steroids is a vital aspect of preventing general and ophthalmic complications.

In conclusion, steroid pulse therapy at the disease onset is of great therapeutic importance in preventing ocular complications. Although both SJS and TEN are self-limiting diseases, appropriate intervention during the acute stage holds great promise for the prevention of corneal epithelial stem-cell loss and corneal and conjunctival cicatricial changes. An appropriate and prompt diagnosis followed by the administration of high-dose corticosteroids may improve the visual prognosis of these 2 devastating diseases.

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LABORATORY INVESTIGATION

The Relationship Between Preoperative Clinical Scores and Immunohistological Evaluation of Surgically Resected Tissues in Chronic Severe Ocular Surface Diseases

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Abstract

Purpose: To clarify the relationship between clinical symptoms and histological status in patients with ocular cicatricial pemphigoid (OCP) and Stevens-Johnson syndrome (SJS).

Methods: Clinical symptoms of four OCP and eight SJS patients in the chronic phase were scored with our recently proposed grading system. The histological status of the pannus tissue removed from the corneal surface during surgery was investigated using immunohistological techniques.

Results: All participants showed total loss of the palisades of Vogt and conjunctivalization of the entire corneal surface. All pannus tissues expressed the conjunctival epithelium marker CK4/13. The pannus tissue in clinically keratinized SJS expressed skin epidermal major cytokeratins, but the tissues of non-keratinized SJS did not.

Conclusions: Clinical observation and the use of our recently proposed grading system agreed with the immunohistological status with respect to keratinization, cell proliferation, and corneal/conjunctival cell typing. These findings facilitate our understanding of the pathogenesis of OCP and SJS, and will hopefully contribute to the development of future treatment strategies and improve predictions of the postoperative prognosis of ocular surface reconstruction in patients with OCP and SJS. **Jpn J Ophthalmol** 2010;54: 66–73 © Japanese Ophthalmological Society 2010

Keywords: clinical score, histopathology, keratinization, ocular cicatricial pemphigoid (OCP), Stevens-Johnson syndrome (SJS)

Introduction

Cicatrizing ocular surface disease is clinically defined by symblepharon, conjunctival invasion, and stem cell deficiency. Stevens-Johnson syndrome (SJS) is a sudden-onset, life-threatening systemic disorder that commonly affects the skin and mucous tissues, including the conjunctiva and cornea.[1] Ocular cicatricial pemphigoid (OCP) is another type of devastating ocular surface disorder that generally affects women over 60 years of age and is characterized by a slowly progressing cicatrization of the ocular surface with minimal inflammation.[1] These alterations may result from destructive changes in tissue organization and a total loss of the corneal epithelial stem cells presumably residing at the corneal limbus;[2] the cornea is often covered by the conjunctiva, resulting in a prominent loss of vision.

Sotozono et al.[3] established methods for classifying and grading the severity of ocular involvement in cases of SJS. Those methods are also useful for the discussion of OCP and chemical injury, as well as for predicting treatment outcomes. The severity of each clinical symptom can be elucidated through the use of this grading method, and a unified grading system for these diseases can thus be established for use in every medical institution. However, no

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reports have compared the scores of this new grading system with observations from immunohistological examination of the pannus tissues from many chronic and extremely severe cases of SJS and OCP. In particular, pannus tissues that have been clinically divided into keratinized and nonkeratinized groups have never before been investigated by detailed immunohistological techniques for the expression of cytokeratins and keratinization-related proteins.

In this study, to clarify the relationship between the clinical status and the histological status of the patients' corneas, the clinical symptoms of four OCP and eight SJS patients in the chronic phase were scored with the grading system established by Sotozono et al.,[3] and the histology of the pannus tissue was investigated by immunohistological techniques.

Methods

Participants

This research was approved by the Committee for Ethical Issues on Human Research of Kyoto Prefectural University of Medicine and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after they had received a detailed explanation of the procedures. The participants comprised four OCP patients (four women; 68.5 ± 24.4 years), eight SJS patients (three men and five women; 44.8 ± 18.8 years), and ten control patients (four men and six women; 67.7 ± 3.8 years). Given that clinically observed keratinization was one of the indexes of the severity of the disease on the ocular surface, we separated the SJS patients into two groups: four with nonkeratinized SJS (SJS K–) (two men and two women; 39.0 ± 29.1 years); and four with keratinized SJS (SJS K+) (one man and three women; 50.8 ± 14.7 years).

Corneal pannus tissues of the OCP and SJS patients were obtained during surgery for transplantation of corneal or oral mucosal epithelial sheets cultivated on amniotic membrane onto the denuded corneas of the patients. As controls, conjunctival tissues were also obtained from patients undergoing conjunctivochalasis operations.[4-6]

Scoring of Clinical Findings

The ocular abnormalities of the patients, especially those of the corneal surface, were evaluated using a standard slit-lamp biomicroscope and scored. The severity of each patient's conjunctivalization, keratinization, and symblepharon was scored from 0 to 3 according to the area of each pathological region in proportion to that of the whole cornea: 0, none; 1, <25%; 2, >25% and <50%; and 3, >50%. The severity of corneal opacification was also graded from 0 to 3: 0, clear cornea with iris details clearly visualized; 1, partial obscuration of the iris details; 2, iris details poorly seen with the pupil margin just visible; and 3, complete obscuration of iris and pupil details. Loss of the palisades of Vogt (POV) was graded from 0 to 3 according to the extent of loss of the limbal POV in proportion to the whole cornea: 0, none; 1: <50%; 2, >50%; and 3, total loss. This grading system has been previously described in detail.[3]

Immunofluorescence Analysis

Tissues were embedded in OCT compound (Tissue-Tek; Sakura Finetechnical, Tokyo, Japan) and snap frozen in liquid nitrogen. The 6-μm sections were placed on glass slides and subjected to a standard indirect immunofluorescence analysis. In brief, the sections were fixed with Zamboni's fixative or acetone at 4°C for 10 min. The sections were then immersed in blocking solution (1% bovine serum albumin; Nacalai Tesque, Kyoto, Japan) in 0.01 M phosphate-buffered saline (PBS) for 60 min. Then, primary antibody solutions (Table 1) were applied to the sections, as were normal mouse IgG1, IgG2a, IgG2b (Dako Cytomation, Kyoto, Japan), rabbit IgG (Dako Cytomation), or goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as negative controls. After a 60-min incubation, the sections were washed three times for 5 min each time with 0.01 M PBS, and the fluorescent secondary antibody solutions (Alexa 488-labeled anti-mouse IgG, anti-rabbit IgG, or anti-goat IgG; Invitrogen, Carlsbad, CA, USA) were applied to the sections. After another 60-min incubation, the sections were washed three times for 5 min each time with 0.01 M PBS and mounted with medium containing 3% antiphotobleaching reagent (DABCO; Wako Pure Chemical Industries, Osaka, Japan). Fluorescent images of the sections were taken through a microscope with a chilled CCD camera (DP50; Olympus, Tokyo, Japan). Unless otherwise stated, all incubations were performed at room temperature.

Data Analysis

Each fluorescent image, other than those of cell nuclei stained with Ki67, was qualitatively scored from 0 to 3 (0, negative; 1, faint; 2, moderate; 3, intense) in a masked fashion by three researchers, and statistical significance was evaluated using the Mann-Whitney *U* test. Ki67-positive cells in the epithelium were counted and normalized relative to the length of the observed area in three sections of each specimen. Statistical significance was evaluated using the two-tailed Welch test.

Results

The clinical information of the participants is summarized in Table 2. The four patients with OCP and the eight patients with SJS showed total corneal stem cell deficiency (loss of POV, score 3) and entire conjunctival invasion (conjunctivalization) of the corneal surface (score 3). Their best-corrected visual acuity (BCVA) ranged from hand motion (HM) to 0.03. All participants required ocular

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Table 1. List of antibodies used in this study

Group	Antigen	Dilution	Type of antibody	Immunized animal	Company*	Annotation
Cytokeratins						
	CK1	×40	MO	M	Novocastra	Major cytokeratin in skin epidermis
	CK3	×50	MO	M	PROGEN	Major cytokeratin in cornea
	CK4	×200	MO	M	Novocastra	Major cytokeratin in nonkeratinizing mucosal epithelium
	CK6	×40	MO	M	Novocastra	Expressed at wound healing or hypeproliferative situation
	CK10	×100	MO	M	Novocastra	Major cytokeratin in skin epidermis
	CK12	×100	PO	G	Santa Cruz	Major cytokeratin in cornea
	CK13	×200	MO	M	Novocastra	Major cytokeratin in nonkeratinizing mucosal epithelium
	CK16	×40	MO	M	Novocastra	Expressed at wound healing or hyperproliferative situation
	CK17	×20	MO	M	Novocastra	Expressed at wound healing or hyperproliferative situation
Proliferation						
	Ki67	×75	MO	M	Dako	Cell proliferation marker
Keratinization-related proteins						
	TGase1	×20	MO	M	Biogenesis	Enzyme that catalyzes the cross-linking of cornified envelope component proteins
	Involucrin	×200	MO	M	Novocastra	Component protein of cornified envelope
	SPRR2A	×4000	PO	R	Apotech	Component protein of cornified envelope
	Filaggrin	×400	MO	M	Biogenesis	Serves as matrix for keratin 1/10 aggregation
	Loricrin	×2000	PO	R	Cvance	Component protein of cornified envelope
Infiltrating cells						
	Neu. Elastase	×100	MO	M	Dako	Marker of neutrophil
	CD4	×100	MO	M	Dako	Marker of helper T cell
	CD3	×100	MO	M	Dako	Marker of pan T cell
	CD8	×50	MO	M	Dako	Marker of suppressor/killer T cell
	CD68	×200	MO	M	Dako	Marker of macrophage
	LFA-1	×100	MO	M	Dako	Marker of T cell, B cell, macrophage, neutrophil elastase
	HLA-DR	×100	MO	M	Dako	Marker of macrophage, Langerhans cell

MO, monoclonal; PO, polyclonal; M, mouse; R, rabbit; TGase1, transglutaminase-1; SPRR2A, small proline-rich protein 2A.
*From which antibodies were purchased: Novocastra, Novocastra Laboratories, Newcastle, UK; Dako, Dako Cytomation, Kyoto, Japan; Biogenesis, Biogenesis, Poole, UK; Apotech, Apotech, Firenze, Italy; Covance, Covance, Princeton, NJ, USA; Santa Cruz, Santa Cruz Biotechnology, Santa Cruz, CA ,USA).

surface reconstruction, but the epithelial condition (nonkeratinized or keratinized), opacification, and symblepharon varied according to preoperative disease severity. All OCP participants showed chronic cicatrization resulting from a shortening of the conjunctival fornix, formation of symblepharon, and opacification (Fig. 1A). The majority of the SJS patients showed severe opacification due to subepithelial fibrosis. However, the score for keratinization varied from 0 to 3 depending on the severity of tear deficiency. Four of the eight SJS patients had severe cicatricial phase SJS without epithelial keratinization (Fig. 1B), and the other four had marked epithelial keratinization (Fig. 1C).

In all OCP, SJS, and control (conjunctivochalasis) conjunctival epithelia, expression of the CK4/13 pair was found at a similar staining intensity in every layer (Figs. 2A, 3A). CK3/12 was expressed in some areas of the epithelium in one OCP and two SJS K– patients (Figs. 2A, 3B). In SJS K+ epithelia (Fig. 3B-4), the staining on the superficial epithelium was nonspecific, as was confirmed by a control experiment using nonimmunized goat IgG.

Transglutaminase 1 (TGase1) expression was significantly increased in OCP and SJS K– and SJS K+ patients as compared to the control patients ($P < 0.05$) (Fig. 2A). Most control patients also exhibited expression of TGase 1, although the staining intensity was fairly faint. Immunostaining analysis revealed that involucrin expression was significantly increased in the OCP and SJS K+ patients as compared to the control patients ($P < 0.05$) (Figs. 2A, 3C). Expression of small proline-rich protein 2A (SPRR2A) was not found in the control conjunctival epithelium but was found in all layers of the epithelium in one OCP, two SJS K–, and four SJS K+ patients. Expression of filaggrin was not found in either control or OCP or SJS K– patients, but it was found in three SJS K+ patients (Figs. 2A, 3D). Those three patients exhibited strong positive staining in the upper half of the epithelial layers (Fig. 3D-4). Expression of loricrin was not found in either control or SJS K– patients, but it was found in one OCP and two SJS K+ patients. Those three patients exhibited intensive immunostaining in the upper half of the epithelial layers, similar to that with

Table 2. Clinical data of OCP and SJS patients

	Patient No.	Sex	Age (years)	Duration from onset (years)	BCVA[a]	Conjunctivalization[b]	Opacification[c]	Keratinization[b]	Symblepharon[b]	Loss of palisades of Vogt[d]
OCP										
	OCP #1	F	83	—	HM	3	2	2	1	3
	OCP #2	F	32	—	HM	3	2	2	2	3
	OCP #3	F	79	—	HM	3	3	0	2	3
	OCP #4	F	80	—	HM	3	2	0	3	3
SJS nonkeratinized										
	SJS #1	M	57	13	0.02	3	2	0	0	3
	SJS #2	M	64	30	HM	3	2	0	0	3
	SJS #3	F	14	10	HM	3	3	0	3	3
	SJS #4	F	21	3	HM	3	3	0	0	3
SJS keratinized										
	SJS #5	F	58	30	0.01	3	2	1	1	3
	SJS #6	M	55	30	0.01	3	3	2	1	3
	SJS #7	F	29	7	0.03	3	1	3	3	3
	SJS #8	F	61	29	CF	3	3	3	1	3

OCP, ocular cicatricial pemphigoid; SJS, Stevens-Johnson syndrome; —, no definite duration; HM, hand motion; CF, counting fingers.

[a]Visual acuity is represented as a decimal value.

[b]Symblepharon, keratinization, and conjunctivalization were scored according to the proportion of the area of the cornea with the pathology: 0, none; 1, <25%; 2, >25% and <50%; 3, >50%.

[c]Corneal opacification was graded as follows: 0, clear cornea with iris details clearly visualized; 1, partial obscuration of the iris details; 2, iris details poorly seen with pupil margin just visible; and 3, complete obscuration of the iris.

[d]Loss of the palisades of Vogt (POV) was graded according to the extent of loss of the limbal POV in proportion to the whole cornea: 0, none; 1, <50%; 2, >50%; 3, total loss.

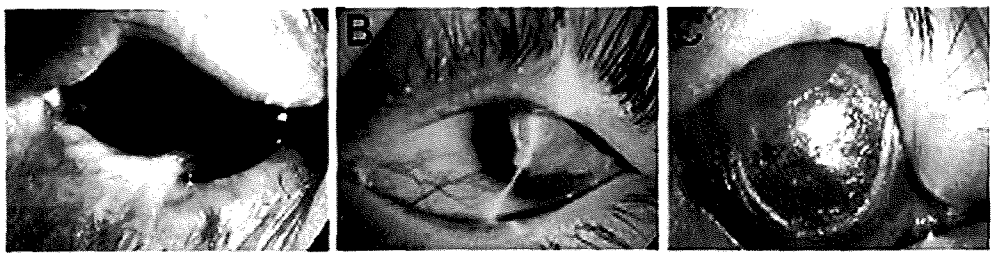

Figure 1A–C. Representative clinical pictures from the ocular surface of ocular cicatricial pemphigoid (OCP) and Stevens-Johnson syndrome (SJS) patients. Clinical appearance of the ocular surface in typical OCP (**A** OCP patient 3) and SJS (**B** SJS patient 3; **C** SJS patient 8) patients enrolled in this study. All patients were in the chronic cicatricial phase and exhibited severe opacification and total conjunctivalization. OCP patient 3 (**A**) and SJS patient 3 (**B**) demonstrated symblepharon formation, and SJS patient 8 (**C**) demonstrated severe keratinization.

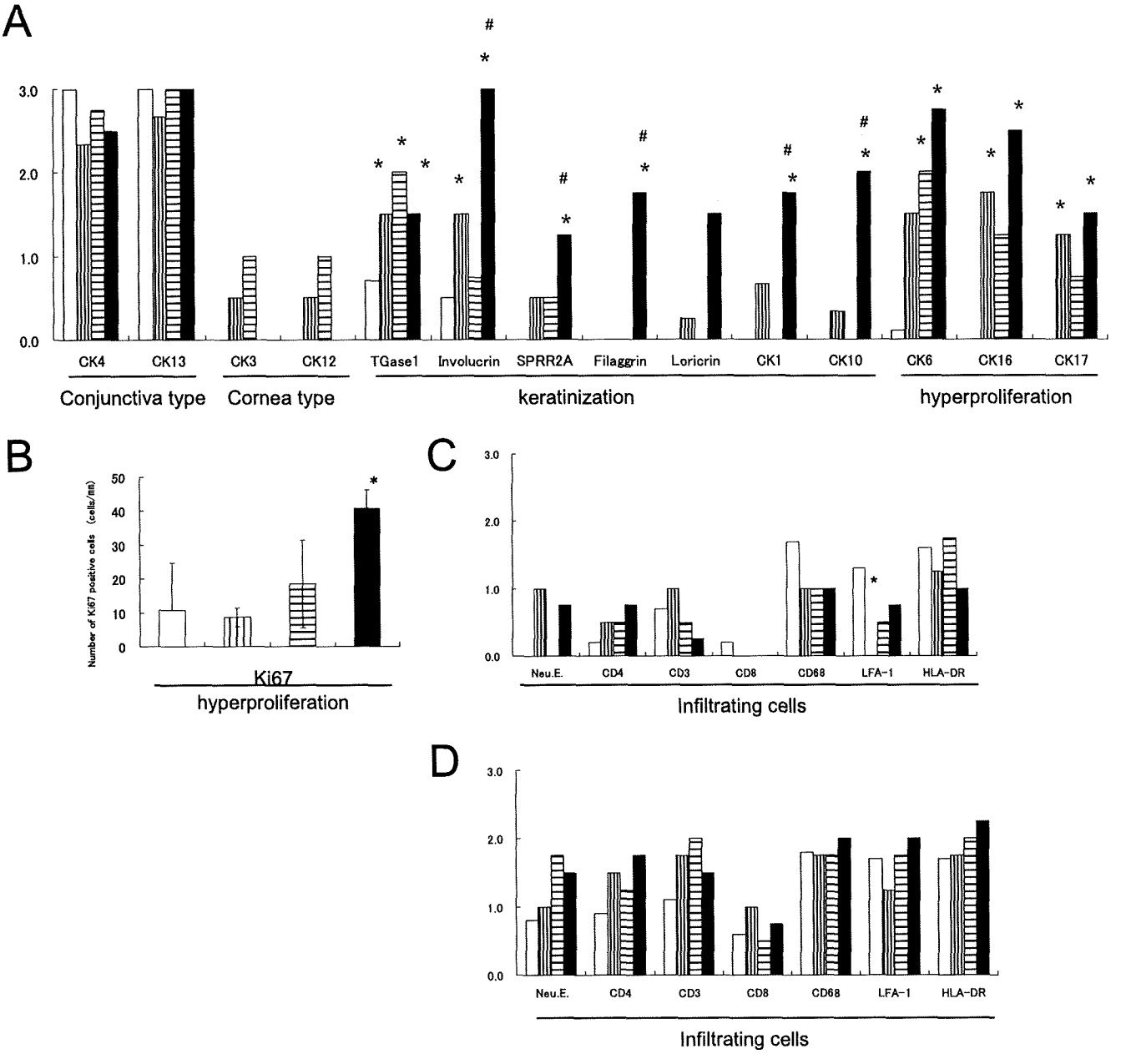

Figure 2A–C. Qualitative and quantitative results from immunofluorescence analysis. Each value represents an average score of the patients of each group. **A** Expression of proteins in the epithelium. **B** Quantitative analysis of Ki67-positive cells. Ki67-positive cells in the epithelium were counted by three masked observers in at least three consecutive images. The length of the basement membrane corresponding to the observed area was measured with Scion Image software (http://www.scioncorp.com), and the number of Ki67-positive cells was normalized relative to the length of the basement membrane in the observed area. Results are expressed as the mean number of Ki67-positive cells ± SD. **C** Infiltrating cells in the epithelium. **D** Infiltrating cells in the subepithelium. *CK*, cytokeratin; *TGase1*, transglutaminase-1; *SPRR2A*, small proline-rich protein 2A; *Neu.E*, neutrophil elastase. □, control (conjunctivochalasis); ▥, OCP; ▤, nonkeratinized SJS (SJS K–); ■, keratinized SJS (SJS K+). **A, C, D** $*P < 0.05$ versus control; $\#P < 0.05$, SJS K– versus SJS K+, Mann-Whitney *U* test. **B** $*P < 0.05$, 2-tailed Welch test.

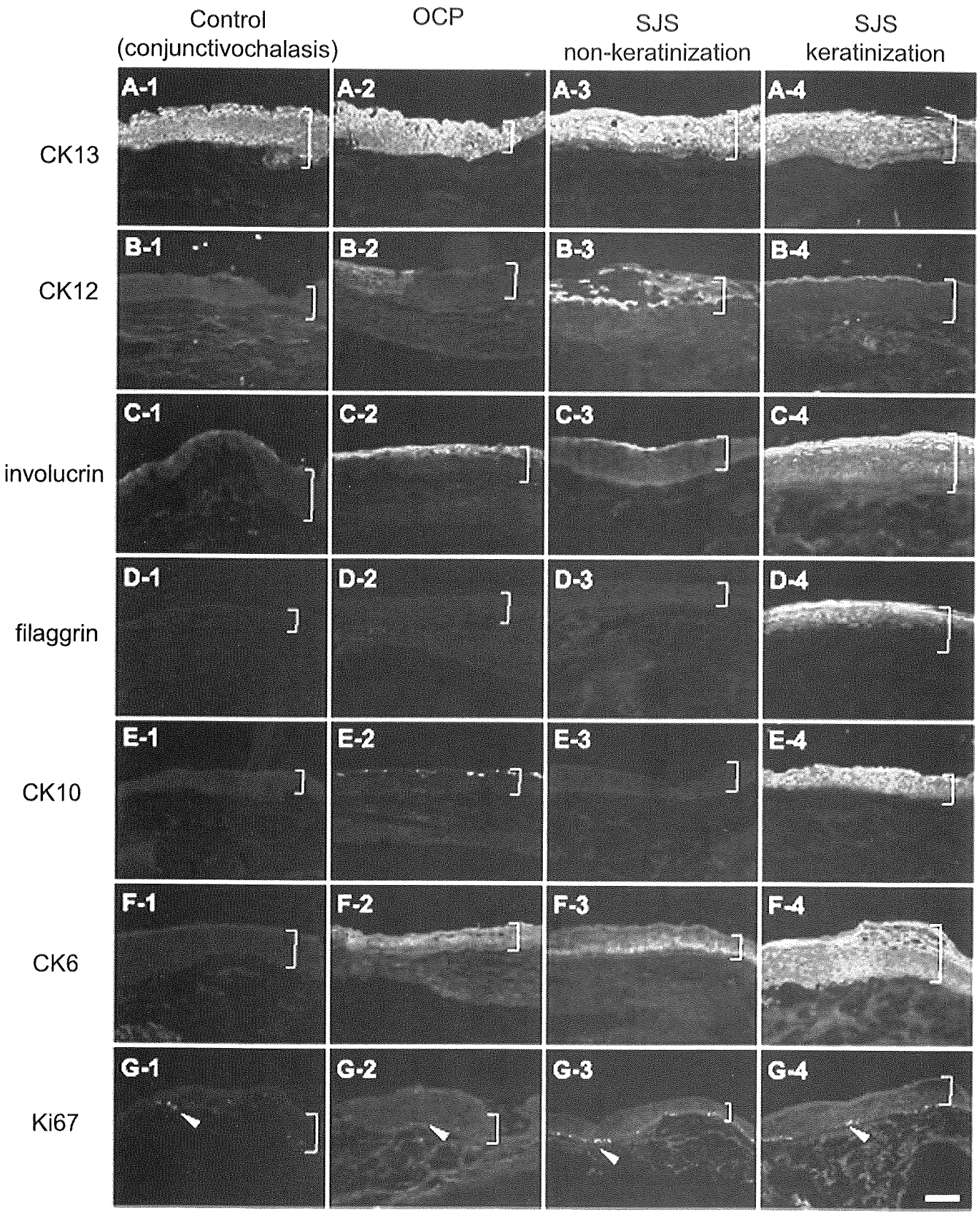

Figure 3A–G. Typical examples of immunofluorescence analysis results. Fluorescent images of the sections were taken with a chilled CCD camera. Each image shows a representative result from each patient group. **A** CK13; **B** CK12; **C** involucrin; **D** filaggrin; **E** CK10; **F** CK6; and **G** Ki67; 1, control (conjunctivochalasis); -2, OCP; -3, SJS K−; and -4, SJS K+. *Square brackets* show the thickness of the epithelium. *Arrowheads* indicate Ki67 antibody-positive cells. Scale bar = 100 μm.

filaggrin. The expression of SPRR2A and filaggrin was significantly different between controls and SJS K+ patients ($P < 0.05$). No expression of CK1 or CK10 was found in either controls or SJS K– patients. However, CK1 was expressed in the upper epithelial layers in two OCP and four SJS K+ patients, and CK10 was expressed in the upper epithelial layers in one OCP and four SJS K+ patients (Figs. 2A, 3E). Expression of these proteins was significantly different between controls and SJS K+ patients ($P < 0.05$). The expression of involucrin, SPRR2A, filaggrin, CK1, and CK10 was significantly different between SJS K– and K+ patients.

Expression of CK6, CK16, and CK17, which are all thought to be hyperproliferation markers, was rare in the control samples but was seen in most OCP and SJS samples. In addition, expression of all three of these proteins was significantly different between controls and SJS K+ patients, that of CK6 was significantly different between controls and SJS K– patients, and that of CK16 and CK17 was significantly different between controls and OCP patients ($P < 0.05$) (Figs. 2A, 3F). Ki67, a cell proliferation marker,[7,8] was detected in the basal or suprabasal layer of the pannus epithelium in all OCP, SJS, and control patients (Fig. 3G). An significantly increased number of Ki67-positive (proliferating) cells in the epithelium were found in both SJS K– and SJS K+ patients ($P < 0.05$, compared with controls) (Fig. 2B).

The number of neutrophil elastase-positive cells and CD4-positive cells was increased in the epithelium and substantia propria of the pannus tissue in both OCP and SJS patients. CD3-positive cells in the substantia propria of the pannus were slightly increased in both OCP and SJS patients. Intraepithelial CD68-positive cells and intraepithelial LFA-1-positive cells were slightly decreased in both OCP and SJS patients. The number of intraepithelial LFA-1-positive cells was significantly different between controls and OCP patients ($P < 0.05$) (Fig. 2C). The increased numbers of neutrophil elastase- and CD4-positive cells in OCP and SJS patients are consistent with the findings of our previous immunohistochemical study.[9] However, in other types of infiltrating cells, no obvious differences were found among OCP, SJS, and control patients, which is inconsistent with our previous study. Current routine regimens for preoperative steroid administration to such patients differ from those of our previous study since it was thought that the inflammation of these chronic pannus tissues was in an inhibited state.

Discussion

We investigated the relationship between our proposed clinical scoring system and the histological status of the corneas of four OCP and eight SJS (four SJS K– and four SJS K+) patients. In all 12 patients, the conjunctiva-type keratin pair CK4/13 was expressed in the pannus tissues covering the corneas; however, most patients lacked the cornea-type keratin pair CK3/12. In addition, the expression level of most of keratinization-related proteins was increased in the pannus epithelia of these patients. Furthermore, hyperproliferation-related keratins and the cell proliferation marker Ki67 were expressed at a higher level in the epithelia of these patients than in those of the control patients. Therefore, these histopathological results show that our clinical scoring system corresponds well to the histopathological status. However, although previous reports have suggested that inflammatory processes may play an important role in the pathogenesis of chronic phase SJS and OCP,[9,10] significant differences were not found in any of our examined markers for various types of infiltrating cells between these patients and the control patients. Topical corticosteroid administration, as well as genetic background and personal circumstances, to these patients may explain this discrepancy between our current results and those of previous reports.

All corneal pannus tissues of the 12 patients expressed the conjunctiva-type keratin pair CK4/13, whereas the cornea-type keratin pair CK3/12 was fully abolished in these tissues. This result suggests that pannus epithelial cells are derived from the surrounding conjunctiva, as previously reported.[11] However, and quite interestingly, two of the SJS K– patients exhibited CK3/12-positive cell clusters in their corneal pannus tissues although they clinically manifested total loss of POV. Possibly, the canonical corneal epithelial cells remained in these patients because their acute inflammatory events were relatively mild and benign. On the other hand, since we have previously reported that CK3/12-positive cell clusters are present as ectopic corneal epithelial cells in the normal conjunctival epithelium,[12] it is possible that the CK3/CK12-positive cells in the two SJS K– patients originated from such ectopically residing corneal epithelial cells.

Keratinization is a well-orchestrated biological process that employs a large number of specialized molecules. During normal keratinization of skin epidermis, filaggrin serves as a matrix for the aggregation of keratin1/10 filaments to establish microfibrils at the cornified layer.[13,14] Simultaneously, the catalytic enzyme TGase1 cross-links loricrin, SPRR2A, and involucrin to form cornified cell envelopes in the lower part of the cornified layer. Although it remains unclear whether the normal epidermal keratinization process is identical to the pathological keratinization process in SJS and OCP, our current results revealed that most of these molecules were increased in the pannus epithelia of these patients. However, the expression pattern of these keratinization-related molecules was not fully coordinated. CK1/CK10, filaggrin, and loricrin were positive only in SJS K+ patients, whereas other keratinization-related molecules were expressed in all SJS and OCP patients as well as in the control subjects. Therefore, these keratinization-related molecules appear to belong to two classes. The first class of molecules (CK1/CK10, filaggrin, and loricrin) seems to be involved in severe or higher level keratinization, and the second class (TGase1, involucrin, and SPRR2A) seems to be involved in mild or lower level keratinization. In addition, the severity of OCP seems to