Allergology International 64 (2015) 379–381

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Letter to the Editor

Drugs causing severe ocular surface involvements in Japanese patients with Stevens–Johnson syndrome/toxic epidermal necrolysis

Dear Editor,

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions often affecting mucosal tissues such as ocular surface, oral cavity, and genitals.[1,2] The severe ocular surface disorders include pseudomembrane formation and epithelial erosion in the acute phase, often leading to permanent impairment or loss of vision.[3]

We recruited Japanese patients with SJS/TEN through participating universities and hospitals and via the nationwide case collection network from June 2006 to June 2013.[4,5] The study was approved by the institutional review boards of all participating institutions, and written informed consent was obtained from all the patients (patient anonymity was preserved using the approved methods). Clinical information such as patients' background, primary (underlying) disease, symptoms, and administered drugs before the onset of SJS/TEN was also collected. Two experts diagnosed the disease using a standardized case report form, containing the criteria proposed by Bastuji-Garin *et al.*[6] except that the SJS–TEN overlap was categorized as TEN according to the severity criteria currently used in Japan.[7] Drugs that were administered continuously to the patients from 1 day to 2 months before the onset of SJS/TEN were assumed to be the causative agents.

According to the clinical information, the ocular surface involvements were graded as follows: 0, no symptoms; 1, only hyperemia of the bulbar and palpebral conjunctiva; 2, either pseudomembrane formation or a defect/erosion of the conjunctiva/corneal epithelia; and 3, both pseudomembrane formation and a defect/erosion of the conjunctiva/corneal epithelia. Grades 0/1 and 2/3 were grouped as mild and severe ocular surface involvements, respectively.

A total of 197 patients with SJS/TEN (97 females, mean age 56.6 ± 22.3 years) were enrolled. The number of probable SJS, SJS, and TEN cases was 23, 115, and 59, respectively. The frequency of severe ocular surface involvement tended to be higher among female and patients younger than 60 years but was not statistically significant (Table 1).

The frequencies of mild and severe ocular surface involvements caused by the drug or the drug group, the number of which was more than 14, were statistically evaluated by Fisher's exact probability test using JMP ver. 7.0.1 (SAS Institute Japan, Tokyo, Japan). As shown in Table 2, patients with SJS/TEN who were treated with cephalosporins or loxoprofen exhibited relatively higher tendencies of experiencing severe ocular surface involvements, but the difference was marginal ($0.05 < p < 0.07$). No differences were observed in the rates of experiencing severe ocular surface involvements in patients with SJS/TEN that is associated with other drugs such as carbamazepine, allopurinol, and quinolones.

On the other hand, we found that patients with SJS/TEN associated with acetaminophen showed a significantly higher rate (55.6%) of experiencing severe ocular surface involvements than those not treated with acetaminophen (27.6%) ($p < 0.01$). Among 27 acetaminophen-administered patients, 16 patients had been diagnosed by DLST, and 10 (62.5%) of them were positive. In the SJS group, the rate of acetaminophen-associated severe ocular involvements (7/36 patients, 19.4%) was significantly higher than those with mild involvements (4/79, 5.1%) ($p = 0.034$, data not shown) while there was no significant association in the case of TEN group, although the skin reaction of TEN is more severe than SJS. These results suggest that the ocular surface would be severely damaged by acetaminophen more frequently than by the other drugs in SJS patients. When acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) were combined as an antipyretic–analgesic (AA) group, the significance was lost ($p = 0.26$).

We divided these AA-associated patients with SJS/TEN into two groups: one group comprised patients taking drugs for common cold and the other comprised patients taking such drugs for conditions other than common cold, before the onset of SJS/TEN. We found that the patients taking these drugs for common cold experienced severe ocular surface involvements at a significantly higher rate (65.4%) than those taking these drugs for conditions other than common cold, such as rheumatoid arthritis (19.5%) ($p < 0.001$). The same was true in the case of acetaminophen ($p < 0.01$). Drugs other than AAs did not show such a tendency ($p > 0.05$, data not shown).

In a previous study by the EuroSCAR group, despite the high relative risk, acetaminophen was regarded as a confounding factor for the assessment of the risk of SJS/TEN because it was often administered concomitantly with other "highly suspected" drugs such as allopurinol and carbamazepine.[8] In the present study,

Table 1
Results of association analysis for patient's background and SJS/TEN with severe ocular involvements.

Category	Factors	Mild	Severe	Severe/Total (%)	Fisher's exact test (p value)
Sex	Male	73	27	27.0	0.22
	Female	62	35	36.1	
Age	≥60	79	28	26.2	0.09
	<60	56	34	37.8	

Number of Japanese SJS/TEN patients with mild/severe ocular involvements and the frequencies of severe cases are shown.

Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2015.05.002

Table 2
Drugs/drug groups and frequencies of ocular surface involvements in Japanese SJS/TEN patients.

	Patients receiving the drug(s)			Patients not receiving the drug(s)			Fisher's exact test (p value)	Odds ratio (95% CI)
	Mild	Severe	Severe/Total (%)	Mild	Severe	Severe/Total (%)		
All patients	135	62	31.5					
Aromatic anti-epileptics (AAE)								
All of AAE	35	17	32.7	100	45	31.0	0.86	
Carbamazepine	11	5	31.3	124	57	31.5	1.00	
Lamotrigine	9	7	43.8	126	55	30.4	0.27	
Anti-hyperuricemia								
Allopurinol	16	10	38.5	119	52	30.4	0.50	
Antibacterial drug								
Quinolones	15	7	31.8	120	55	31.4	1.00	
Proton pump inhibitors	12	3	20.0	123	59	32.4	0.40	
Antibiotics								
All of antibiotics	30	18	37.5	105	44	29.5	0.26	
Cephalosporins	11	11	50.0	124	51	29.1	0.05	2.43 (0.99/5.97)
Antipyretic analgesics (AAs)								
All of AAs	42	25	37.3	93	37	28.5	0.26	
Aspirin	8	7	46.7	127	55	30.2	0.25	
Loxoprofen	13	12	48.0	122	50	29.1	0.07	2.25 (0.96/5.28)
Acetaminophen	12	15	55.6	123	47	27.6	0.0065	3.27 (1.43/7.50)
Patients receiving AAs for treatment of cold[†]	9	17	65.4				0.0002	7.79 (2.55/23.8)
Patients receiving AAs for treatment of other diseases	33	8	19.5					
Acetaminophen for treatment of cold[†]	4	13	76.5				0.0069	13.0 (1.92/88.0)
Acetaminophen for treatment of other diseases	8	2	20.0					

Japanese SJS/TEN patients were categorized by culprit drugs/drug groups.
[†] Judged according to primary (underlying) disease in the filled case report forms (e.g. common cold, acute upper respiratory inflammation, and acute adenoiditis) and/or drug name (e.g. multi-ingredient cold remedy). Mycoplasma pneumoniae or influenza were excluded from this category.

however, 14 of 15 patients with acetaminophen-associated SJS/TEN and severe ocular surface involvement had not taken such drugs together. Therefore, at least among Japanese patients with SJS/TEN with severe ocular surface involvements, acetaminophen is strongly suspected to be a causative drug, particularly when this drug was taken for the treatment of common cold.

In conclusion, we found that 1) patients with SJS/TEN taking acetaminophen showed a significantly higher rate of experiencing severe ocular surface involvements than those taking other SJS/TEN frequently causative drugs such as carbamazepine, allopurinol, and quinolones; 2) the patients taking AAs, including acetaminophen and/or NSAIDs, for the treatment of common cold showed a high frequency of patients with SJS/TEN experiencing severe ocular surface involvements compared with those taking AAs for the treatment of other diseases. These results suggest that not only AAs including cold medicine but also viral infections causing cold-like symptoms play some important roles in the development of severe ocular surface involvements. Note that our recent study showed that cold medicine-associated patients with SJS/TEN with severe ocular complications are associated with certain types of HLA (*HLA-A*02:06* and *HLA-A*44:03*)[9] and/or *IKZF1*.[10] Taken together, these results suggest the existence of unknown unique mechanisms underlying the development of ocular disorders in SJS/TEN caused by AAs. The patients with SJS/TEN taking these drugs for common cold should be taken special care of their eyes and the skin to prevent severe ocular sequelae.

Acknowledgements

The study was supported in part by the Health Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, and JSPS KAKENHI Grant (26293122).

Conflict of interest
The authors have no conflict of interest to declare.

Nahoko Kaniwa [a,g], Mayumi Ueta [b,g], Ryosuke Nakamura [a,*,g], Yoshimi Okamoto-Uchida [a], Emiko Sugiyama [a], Keiko Maekawa [a], Yukitoshi Takahashi [c], Hirokazu Furuya [d], Akiko Yagami [e], Setsuko Matsukura [f], Zenro Ikezawa [f], Kayoko Matsunaga [e], Chie Sotozono [b], Michiko Aihara [f], Shigeru Kinoshita [b], Yoshiro Saito [a]

[a] *Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan*
[b] *Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan*
[c] *Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan*
[d] *Department of Neurology, Kochi Medical School, Kochi, Japan*
[e] *Department of Dermatology, School of Medicine, Fujita Health University, Aichi, Japan*
[f] *Department of Environmental Immuno-Dermatology, Graduate School of Medicine, Yokohama City University, Kanagawa, Japan*

* Corresponding author. Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan.
E-mail address: ryosnak@nihs.go.jp (R. Nakamura).

References

1. Roujeau JC. Stevens–Johnson syndrome and toxic epidermal necrolysis are severity variants of the same disease which differs from erythema multiforme. *J Dermatol* 1997;**24**:726–9.
2. Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern S, Anderson T, et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med* 1995;**333**:1600–7.
3. Power WJ, Ghoraishi M, Merayo-Lloves J, Neves RA, Foster CS. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-

[g] These authors contributed equally to this work.

Johnson syndrome/toxic epidermal necrolysis disease spectrum. *Ophthalmology* 1995;**102**:1669–76.
4. Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, et al. HLA-B*1511 is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Epilepsia* 2010;**51**:2461–5.
5. Tohkin M, Kaniwa N, Saito Y, Sugiyama E, Kurose K, Nishikawa J, et al. A whole-genome association study of major determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Pharmacogenomics J* 2013;**13**:60–9.
6. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch Dermatol* 1993;**129**:92–6.
7. Hashimoto K, editor. *[Ministry of Health, Labour and Welfare Report: A Study for Diagnostic Criteria and Medical Guidelines for Stevens-Johnson Syndrome, Toxic Epidermal Necrolysis, Hypersensitivity Syndrome]*. Ministry of Health, Labour and Welfare, Japan, 2002 (in Japanese).
8. Mockenhaupt M, Viboud C, Dunant A, Naldi L, Halevy S, Bouwes Bavinck JN, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. *J Invest Dermatol* 2008;**128**:35–44.
9. Ueta M, Kaniwa N, Sotozono C, Tokunaga K, Saito Y, Sawai H, et al. Independent strong association of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement. *Sci Rep* 2014;**4**:4862.
10. Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, et al. IKZF1, a new susceptibility gene for cold medicine–related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvements. *J Allergy Clin Immunol* 2015;**135**:1538–45.

Received 18 December 2014
Received in revised form 30 April 2015
Accepted 1 May 2015

AMERICAN ACADEMY OF OPHTHALMOLOGY
The Eye M.D. Association

The Role of Systemic Immunomodulatory Treatment and Prognostic Factors on Chronic Ocular Complications in Stevens–Johnson Syndrome

Dong Hyun Kim, MD,[1,2,] Kyung Chul Yoon, MD, PhD,[3,*] Kyoung Yul Seo, MD, PhD,[4] Hyo Seok Lee, MD,[3] Sang Chul Yoon, MD,[4] Chie Sotozono, MD, PhD,[5] Mayumi Ueta, MD, PhD,[5] Mee Kum Kim, MD, PhD[1,2]*

Purpose: To compare the effect of early systemic immunomodulatory treatment and to identify prognostic factors of chronic ocular complications in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) patients.

Design: Retrospective, comparative, multicenter study.

Participants: Forty-three patients admitted to 1 of 3 University Hospitals (Seoul National University Hospital, Chonnam National University Hospital, and Yonsei University Hospital) with a diagnosis of SJS or TEN who were followed up for at least 6 months in Korea.

Methods: Patients were divided into 5 groups according to systemic immunomodulatory treatment received: systemic steroids (S), intravenous immunoglobulin (IVIG), combined S plus IVIG, systemic pulse steroids (PS), and supportive care only (C). Best-corrected visual acuity (BCVA) and chronic ocular surface complications score (COCS; range, 0–15) at final follow-up were compared among the 5 groups. Prognostic factors at onset (age, gender, causative drugs, initial visual acuities, acute ocular involvement score [range, 0–3], acute systemic involvement score [range, 0–16], systemic steroid dose, IVIG dose, and amniotic membrane transplantation [AMT]) were analyzed to predict final BCVA or COCS using logistic regression or linear regression analysis.

Main Outcome Measures: Best-corrected visual acuity and COCS at final follow-up.

Results: The mean age and follow-up period of the patients was 30.5±21.0 years and 29.1±30.4 months, respectively. The acute systemic involvement score in the IVIG, S plus IVIG, and PS groups was significantly higher than that in the S and C groups ($P < 0.001$). However, final BCVA and COCS were not significantly different between groups, even after statistical adjustment. High COCS (≥8 points) was associated with female gender ($P = 0.012$) and AMT at the acute stage ($P = 0.040$). High acute ocular and systemic involvement scores were associated with worse COCS ($P < 0.001$), and COCS showed good correlation with final BCVA ($R^2 = 0.7101$; $P < 0.0001$).

Conclusions: There were no therapeutic benefits of systemic immunomodulatory treatments in final visual outcome and COCS in SJS and TEN patients. Female gender and acute ocular and systemic involvement scores may be prognostic factors predicting chronic ocular complications. *Ophthalmology 2015;122:254-264 © 2015 by the American Academy of Ophthalmology.*

Supplemental material is available at www.aaojournal.org.

Stevens-Johnson syndrome (SJS), and its more severe variant, toxic epidermal necrolysis (TEN), are acute self-limiting diseases of the skin and mucous membranes that predispose patients to life-threatening complications.[1–4] Ocular complications in SJS and TEN may occur acutely, together with the onset of skin involvement in more than 50% of patients,[5–8] resulting in cicatricial changes and permanent visual impairment.[8–10] Therefore, patients with visual impairment and chronic ocular complications after SJS or TEN may require life-long treatment for ocular complications.

Stevens-Johnson syndrome and TEN are drug-induced immune responses in which $CD8^+$ cytotoxic T cells, soluble Fas ligand, nitric oxide, and tumor necrosis factor-α contribute to keratinocyte apoptosis.[11] There is no well-established treatment for these diseases, nor for the associated ocular complications. Systemic corticosteroids and intravenous immunoglobulin (IVIG) are 2 representative immunomodulatory treatments for SJS and TEN. However, the therapeutic effect of corticosteroids or IVIG in SJS and TEN patients has been controversial.[12–19] Moreover, to our knowledge, there has been no study comparing the effect of several systemic

http://dx.doi.org/10.1016/j.ophtha.2014.08.013
ISSN 0161-6420/14

immunomodulatory treatments on chronic ocular complications in SJS or TEN. In addition, there are only few studies of prognostic factors that could predict chronic ocular complications in SJS or TEN.[20,21] Therefore, we aimed (1) to compare the effect of the administration of systemic immunomodulatory treatments during the acute stages of SJS or TEN on final visual outcome and chronic ocular complications and (2) to identify the prognostic factors that predict worse final visual outcome and chronic ocular complications.

Methods

Subjects and Study Design

We retrospectively reviewed the medical records of the patients who visited the Department of Ophthalmology in 1 of 3 University Hospitals (Seoul National University Hospital, Chonnam National University Hospital, or Yonsei University Hospital) with a diagnosis of SJS or TEN between December 2003 and March 2013. The institutional review board of Seoul National University Hospital approved the study protocol (institutional review board no., 1109-082-378), and the protocol complied with the tenets of the Declaration of Helsinki.

The inclusion criteria were as follows. (1) The patients were diagnosed with SJS or TEN by a dermatologist or a physician. The diagnosis of SJS or TEN in the acute phase was based on the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and the involvement of at least 2 mucosal sites, including the ocular surface. Stevens-Johnson syndrome and TEN were characterized by an epidermal detachment of less than 30% and more than 30% of the body surface area, respectively.[1] (2) The patients had medical records of the acute phase, which were fully accessible to evaluate prognostic factors. (3) The patients were followed up for at least 6 months. (4) The patients did not have a history of other ocular surface disorder or ocular surgery.

Eighty-six eyes of 43 patients (19 men, 24 women; mean age, 30.9±20.9 years; range, 3–66 years) were included in this multicenter study. The age, gender, causative drugs, mean interval between the disease onset and initial systemic treatment, mean interval between acute ocular complication onset and initial systemic treatment, initial visual acuities in the acute phase, follow-up period, acute ocular involvement score (AOS; range, 0–3), acute systemic involvement score (ASS; range, 0–16), systemic steroid dose, IVIG dose, undergoing amniotic membrane transplantation (AMT) in the acute phase, best-corrected visual acuity (BCVA) at final follow-up, and chronic ocular surface complications score (COCS; range, 0–15) were investigated.

To determine the effect of immunomodulatory treatments in early-stage disease, final BCVA (in logarithm of the minimum angle of resolution [logMAR] units) and COCS were compared among the 5 groups based on the systemic immunomodulatory treatments. To determine the prognostic factors, we investigated which factors at onset could be related to final BCVA or COCS by using the following methods. (1) We analyzed the factors related with poor chronic outcome such as final BCVA worse than 20/200 or COCS of 8 or more using logistic regression analysis. (2) We divided the patients into 2 groups based on the factors at onset (age, ≥18 years vs. <18 years; gender, male vs. female; initial diagnosis, SJS vs. TEN; AOS, ≥2 vs. <2; ASS, ≥8 vs. <8; AMT, AMT performed vs. AMT not performed) and compared final BCVA (logMAR) and COCS between the 2 groups using a nonparametric analysis of covariance. Finally, (3) we investigated the relationship of AOS or ASS with the final BCVA or COCS using linear regression analysis.

Early Systemic Treatment during the Acute Phase of Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis

Supportive care using preservative-free artificial tears, topical steroids, and antibiotics was given to all patients in the acute stage. According to the early systemic immunomodulatory treatments, the patients were divided into 5 groups: (1) conventional systemic steroids (S; n = 18), (2) combined conventional S and IVIG (S plus IVIG; n = 15), (3) IVIG only (n = 5), (4) systemic pulse steroid (PS; n = 3), and (5) supportive care only without any systemic treatment (C; n = 3). All the patients received systemic immunomodulatory treatments within 7 days after onset of disease except the patients of group 5 (C). The detailed application dosages of the treatment were as follows. (1) Conventional S initially were administered intravenously or orally at a mean dosage of 1.0 mg/kg daily ranging from 0.17 to 1.7 mg/kg daily (the dosage of the various kinds of steroids was converted into the dosage of the prednisolone equivalent) for 2 to 11 consecutive days (mean, 5.65 days) and tapered. (2) Pulse steroid dosage was 250 to 1000 mg/day for 3 to 4 consecutive days. (3) A total dose of 0.74 to 7.5 g/kg IVIG (mean, 2.53 g/kg) was administered continuously within 5 days after admission.

Acute Ocular and Systemic Involvement Scores

Acute ocular involvements of patients were scored from 0 to 3, depending on conjunctival hyperemia, pseudomembrane formation, and corneal epithelial erosion by corneal specialists (Table 1). The definition of corneal epithelial erosion included both full-thickness corneal epithelial defect and diffuse superficial punctate erosion. Diffuse superficial punctate erosion includes area 2 and density 3, area 3 and density 2, or area 3 and density 3.[9,22] Acute systemic involvements were scored from 0 to 16, depending on oral or genital erythema, degree of epidermal detachment, fever, respiratory disturbance, total necrosis of epidermis, liver dysfunction, anemia, elevated serum C-reactive protein concentrations, kidney dysfunction, and pneumonia (Table 2). Each component of acute systemic involvement was determined based on severity of illness score for TEN, Japanese acute systemic index score by the Japanese Research Committee on Severe Cutaneous Adverse Reaction, or previously described clinical findings.[23,24] Acute ocular and systemic involvement scores (AOS and ASS) of more than 2 and 8 points, respectively, were defined as severe AOS and ASS. Figure 1 shows one of the representative manifestations of AOS.

Table 1. Acute Ocular Involvement Score

Acute Ocular Manifestations	Scoring
No involvement	0
Conjunctival hyperemia	1
Pseudomembrane formation or corneal epithelial erosion	2
Pseudomembrane formation and corneal epithelial erosion	3

Amniotic Membrane Transplantation

If severe acute ocular manifestation was identified and continued, amniotic membrane was transplanted transiently within 3 months after the onset of SJS or TEN. Amniotic membrane transplantation was performed as described by John et al.[25]

Visual Outcome and Chronic Ocular Surface Complication Score at Final Follow-up

Best-corrected visual acuity and COCS at final follow-up were used to assess chronic ocular complications in SJS or TEN patients as main outcomes. Final BCVA was obtained before corneal or limbal transplantation. Poor final BCVA was defined as less than 20/200. Chronic ocular surface complications score (range, 0–15) was adapted with modifications based on the grading system established by Sotozono et al.[9] Chronic ocular surface complications score included scoring of conjunctival hyperemia, decreased tear volume, eyelid involvement, corneal involvement, limbal deficiency, and symblepharon formation, and COCS included scoring depending on increased involvement area or severity of the above-listed factors (Table 3). Decreased tear volume was defined as less than 5 mm of moisture on the filter paper within 5 minutes by Schirmer's test. Severe COCS was defined as 8 points or more (Fig 2).

Statistical Analysis

Data are presented as the mean ± standard deviation. All data were analyzed using SPSS Statistics software version 21 (SPSS, Inc., Chicago, IL). Kruskal-Wallis and Fisher exact tests were used to compare patient demographics and the effects of systemic treatments on chronic ocular manifestations among the 5 treatment groups. Nonparametric analysis of covariance was used additionally to adjust the differences of several groups. Univariate and multivariate logistic regression analyses were used to investigate prognostic factors related with poor final visual outcome (BCVA <20/200) and high COCS (≥8 points). Multivariate logistic regression analysis was performed with variables whose P value was less than 0.1 in univariate analysis. Linear regression analyses were used for the evaluation of the relationships among AOS, ASS, and chronic ocular complications and between final BCVA (logMAR) and COCS. Statistical significance was defined as $P < 0.05$. The significant P value limit was modified according to Bonferroni's correction method to address problems caused by multiple comparisons.

Results

Patient Characteristics and Identifiable Causes of Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis

The characteristics of the patients and identifiable causes of SJS or TEN are summarized in Table 4 and Table 5 (available at www.aaojournal.org). Mean follow-up period was 29.1±30.4 months (range, 6–131 months). Mean AOS was 2.21±0.88. Acute ocular involvement score was 1 point in 20 eyes (23.3%), 2 points in 22 eyes (25.6%), and 3 points in 42 eyes (48.8%). Acute ocular involvement score was not different between SJS and TEN patients (SJS, 2.25±0.87; TEN, 2.13±0.90; $P = 0.562$, Mann–Whitney U test). Mean ASS was 7.48±2.61. The ASS in TEN patients was higher than that in SJS patients, as expected (SJS, 6.46±2.35; TEN, 9.37±1.93; $P < 0.001$, chi-square test). The most common causative drug was nonsteroidal anti-inflammatory drugs (NSAIDs; 15 patients [40.5%]), followed by antibiotics (7 patients [18.9%]).

Table 2. Acute Systemic Involvement Score

Systemic Involvement	Scoring
Erythema	0–4
Oral erosive lesions with bloody scales	1
Labial erosive lesions with bloody scales	1
Oral or labial erosive lesions only	1
Genital involvement	1
Blisters, epidermal detachment, erosions (% of total body surface area; selection of 3 items below)	0–3
>30%	3
10%–30%	2
≤10%	1
High fever (≥38° C)	1
Respiratory disturbance	1
Total necrosis of epidermis	1
Liver dysfunction (IU/l; selection of 2 items below)	0–2
ALT or AST >100	2
40<ALT ≤100 or 40<AST ≤100	1
Anemia (hemoglobin <10 g/dl)	1
Serum CRP ≥5 mg/dl	1
Kidney dysfunction (serum blood urea nitrogen >10 mmol/l)	1
Pneumonia (pneumonic infiltration in chest radiograph film)	1
Total	16

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein.

Patients' Characteristics According to Early Systemic Immunomodulatory Treatment

Table 6 summarizes the patients' characteristics according to treatment. Age, gender, initial visual acuity (logMAR), and AOS showed no differences between the groups. However, there were significant differences in the proportion of TEN to SJS, ASS, and mean interval between acute ocular complication onset and initial treatment. The relative proportion of TEN and ASS of 8 points or more was higher in group 2 (S plus IVIG), group 3 (IVIG), and group 4 (PS) than in groups 1 (S) and 5 (C; $P < 0.005$ for all comparisons, Fisher exact test). Group 5 (C) showed a significantly longer interval between the onset of acute ocular complications and initial treatment than others ($P < 0.005$, Mann–Whitney U test). Total systemic steroid dose in group 4 (PS) was higher than that in group 1 (S; $P < 0.005$, Mann–Whitney U test). The total dose of IVIG (grams per kilogram) was similar between groups 2 (S plus IVIG) and 3 (IVIG only).

Final Visual Outcome and Chronic Ocular Surface Complications Score According to Early Systemic Immunomodulatory Treatment

Mean final BCVA (logMAR) and mean COCS were 0.40±0.86 and 3.45±3.66, respectively. The BCVA and COCS at final follow-up were not significantly different among the 5 groups

(Table 7). Because a larger proportion of TEN and higher ASS may be related to poor chronic ocular complications in groups 2, 3, and 4, we adjusted all variables listed in Table 6 except initial visual acuity and steroid or IVIG dosage. However, final visual outcome and COCS were not significantly different despite adjustment to patient demographics using a nonparametric analysis of covariance (Table 7).

Subgroup Analysis of the Effect of Variables at the Acute Stage on Chronic Ocular Complications

Interestingly, we found a significant relationship of final BCVA or COCS with several factors such as female gender, severe AOS (≥2 points), and ASS (≥8 points; Table 8). Amniotic membrane transplantation at the acute stage also was related to high COCS (poor chronic outcome). Considering that AOS of all the patients who underwent AMT (n = 12) was 3 points, which was significantly higher than in the no AMT group (n = 74; 2.08±0.89; $P = 0.031$, Mann–Whitney U test), the relationship of AMT to high COCS seemed to be caused by high AOS. To avoid the confounding effect of AOS on the relationship between AMT and COCS, we compared the effect of AMT only in the patients whose AOS was 3 (n = 42; Table 9, available at www.aaojournal.org). This comparison revealed no differences of final BCVA and COCS in those patients, regardless of AMT. This implies that high AOS may be a more critical prognostic factor to predict chronic complication rather than the history of AMT per se. Adults (18 years or older) showed no differences in final BCVA and COCS compared with children (younger than 18 years).

Prognostic Factors Related to Poor Final Visual Outcome or High Chronic Ocular Complications Score

Prognostic factors related to poor final visual outcome (BCVA <20/200) and high COCS (≥8 points) using logistic regression analysis are shown in Table 10. Amniotic membrane transplantation had a significant relationship with poor final visual outcome (odds ratio [OR], 4.125 in univariate analysis [$P = 0.048$]; OR, 4.418 in multivariate regression [$P = 0.050$]) and severe COCS (OR, 6.9 in univariate analysis [$P = 0.012$]; OR, 8.428 in multivariate regression [$P = 0.012$]). Female gender showed a marginally significant relationship with poor final visual outcome (OR, 4.737 in univariate analysis [$P = 0.055$]; OR, 4.975 in multivariate regression [$P = 0.053$]) but a significant relationship with high COCS (OR, 18.52 in univariate analysis [$P = 0.049$]; OR, 21.564 in multivariate regression [$P = 0.012$]). Systemic immunomodulatory treatments were not associated with final visual outcome and COCS.

Relationship of Acute Ocular and Systemic Involvement with Final Visual Outcome and Chronic Ocular Complications Score

Figure 3 shows that final BCVA and COCS were affected significantly by acute ocular and systemic involvement. Acute ocular involvement score and ASS had a larger correlation with COCS (AOS: $R^2 = 0.2295$, $P < 0.0001$; ASS: $R^2 = 0.2071$, $P < 0.0001$) than with final BCVA (AOS: $R^2 = 0.1037$, $P = 0.0025$; ASS: $R^2 = 0.0898$, $P = 0.0051$). In addition, there was a significant correlation between acute ocular and systemic involvement ($R^2 = 0.3452$, $P < 0.001$).

Relationship Between Final Visual Outcome and Chronic Ocular Complications Score

To validate COCS as a clinically relevant assessment tool, we assessed the correlation of COCS with the final BCVA. There was a good correlation between final BCVA and COCS ($R^2 = 0.7101$, $P < 0.0001$; Fig 4).

Discussion

Our study revealed that none of the systemic immunomodulatory treatments showed better therapeutic benefits than supportive care in final visual outcome and COCS. As prognostic factors, female gender, acute ocular and systemic involvement, and AMT were related to poor BCVA and high COCS. AMT may be biased by the fact

Table 3. Chronic Ocular Surface Complications Score

Chronic Ocular Complications	Severity or Involvement Area	Scoring
Chronic conjunctival hyperemia		1
Decreased tear volume (Schirmer strip test ≤1 mm/min)		1
Lid involvement	Trichiasis	1
	Distichiasis	1
	Severe meibomian gland dysfunction	1
Corneal involvement	Superficial punctate keratitis	1
	Corneal thinning	1
	Corneal opacity	1
Limbal deficiency	Partial corneal neovascularization	1
	Near total corneal neovascularization with persistent corneal epithelial defect	2
	Total conjunctivalization	3
Symblepharon formation	1 quadrant involved	1
	2 quadrants involved	2
	3 quadrants involved	3
	4 quadrants involved	4
Total		15

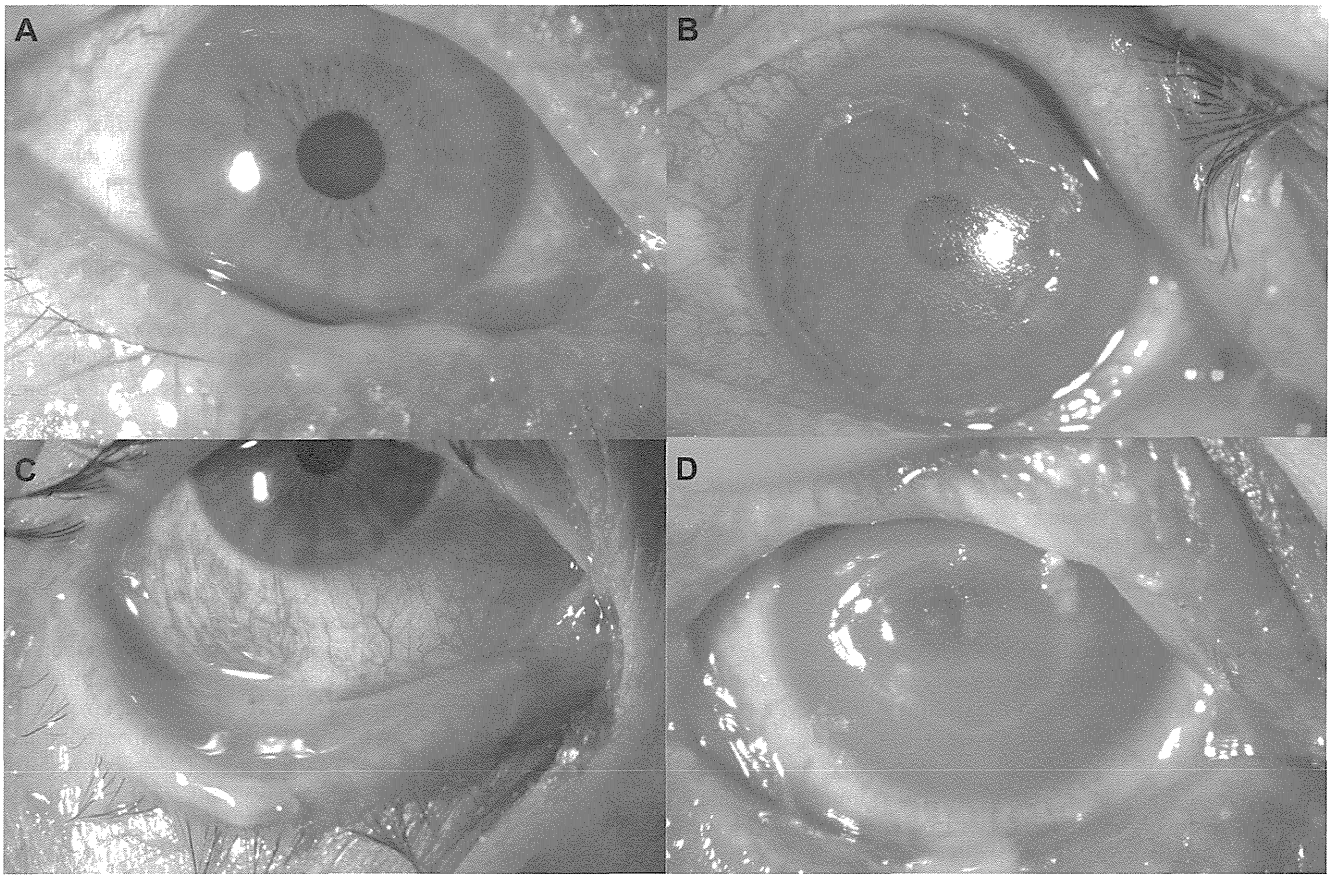

Figure 1. Photographs showing acute ocular involvement score: (**A**) simple conjunctival hyperemia (1 point); (**B**) conjunctival hyperemia with corneal epithelial erosion (2 points); (**C**) conjunctival hyperemia with pseudomembrane formation (2 points); (**D**) conjunctival hyperemia with corneal persistent epithelial defect and pseudomembrane formation (3 points).

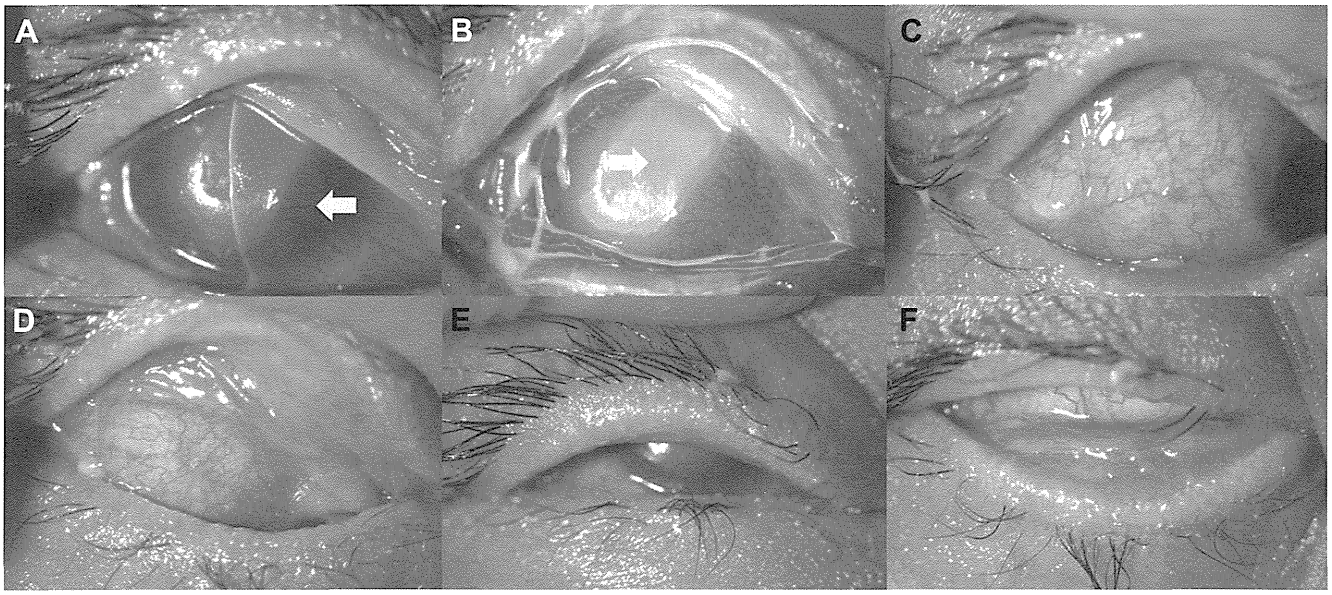

Figure 2. Anterior segment photographs of patients whose chronic ocular surface complications score (COCS) was 14 points (8 months after onset of the disease): (**A**) corneal thinning with opacity and near-total corneal neovascularization (yellow arrow); (**B**) superficial punctate keratitis with persistent corneal epithelial defect (yellow arrow); (**C**) diffuse conjunctival hyperemia; (**D**) symblepharon formation; (**E**) severe meibomian gland dysfunction; and (**F**) trichiasis and distichiasis.

Table 4. Characteristics of Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis Patients in the Study

Characteristic	Data
Mean age at disease onset (yrs)	30.5±21.0
No. of adults/children*	27/16
Gender (male/female)	19/24
SJS/TEN	28/15
Mean AOS	2.21±0.88
Mean ASS	7.48±2.61
Mean interval	
Onset of disease until systemic treatment (days)	3.88±4.03
Onset of acute ocular complication until systemic treatment (days)	3.26±3.56
Onset of disease until acute ocular complications (days)	2.60±3.57
Mean follow-up (mos)	29.1±30.4
Initial visual acuities at onset of SJS/TEN (logMAR)	0.51±0.60

AOS = acute ocular involvement score; ASS = acute systemic involvement score; logMAR = logarithm of the minimum angle of resolution; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis.
*Younger than 18 years.

that early AMT is required only in patients with severe acute ocular complications. Actually, after adjustment by regrouping the patients with maximum AOS, AMT per se did not show a beneficial effect on chronic ocular complications.

There is no consensus about systemic immunomodulatory treatments in SJS or TEN. Corticosteroids or IVIG have been used as first-line therapies, yet the systemic role of those in SJS or TEN is controversial,[13,15–19,26–33] although corticosteroids decrease inflammatory cytokine level[28,29] and IVIG can inhibit keratinocyte apoptosis.[34] Few studies have investigated the effect of early systemic immunomodulatory treatments on chronic ocular damage. A small case series indicated that PS therapy in the acute phase prevented ocular complications,[35] but others indicated that systemic steroid or IVIG therapy did not limit ocular damage.[36,37] In this study, no systemic immunomodulatory treatments presented benefits on final visual outcome and chronic ocular complications. These results corroborate with the previous reports showing no benefits of systemic treatments on mortality. Our previous study did not prove the benefits of each early immunomodulatory treatment compared with conservative treatment either, although it suggested early intervention with steroid or IVIG therapy may improve BCVA compared with late intervention with steroid or IVIG therapy.[38] Considering that the mean IVIG dose was more than 2 g/kg and a high dosage of the PS therapy was used in the study, the pharmacologic effect of immunomodulatory treatments was probably not insufficient. Given that ASS is associated with chronic ocular complications, we cannot completely exclude the possibility that the difference in ASS among the treatment groups may have biased the immunomodulatory treatment effect. A prospective, randomized study is ethically unrealizable, and the limitation of a retrospective study thus is acceptable.

With regard to prognostic factors of chronic ocular complications, AOS and ASS were associated significantly with chronic ocular complications. This finding was in agreement with the reports of Arstikaitis[21] and Gueudry et al.[20] Meanwhile, Yip et al[7] demonstrated that the severity of acute ocular complications was not a risk factor for late ocular complications. The results achieved by Gueudry

Table 6. Patients' Characteristics According to Systemic Treatment

	Group 1 (S)	Group 2 (S plus IVIG)	Group 3 (IVIG)	Group 4 (PS)	Group 5 (C)	*P* Value
Patients (eyes)	18 (36)	14 (28)	5 (10)	3 (6)	3 (6)	
Age*	33.2±17.0	32.1±25.9	19.4±11.5	37.0±26.7	18.3±19.1	0.156
Gender (male/female)†	16/20	14/14	2/8	2/4	4/2	0.387
SJS/TEN*	30/6	14/14‡	4/6‡	2/4‡	6/0	**0.001**
Initial visual acuity (logMAR)*	0.50±0.55	0.66±0.69	0.45±0.36	0.16±0.16	0.47±0.35	0.130
Mean interval (days)*,§	2.8±0.4	3.0±0.8	2.0±0.9	5.0±1.3	7.7±1.8	**0.029**
Acute ocular involvement score (≥2/<2)†	23/13	23/5	5/5	4/2	4/2	0.198
Acute systemic involvement score (≥8/<8)†	10/26	18/10‡	8/2‡	6/0‡	2/4	**<0.001**
AMT (not performed/performed)†	35/1	21/7	8/2	6/0	4/2	0.127
Initial first week systemic steroid dosage‖ (mg/kg)*	6.72±4.28	7.65±4.26	—	37.96±20.91	—	**<0.001**
Total systemic steroid dose‖ (mg/kg)*	11.02±5.83	28.42±38.73	—	41.16±18.44	—	**0.003**
Total IVIG dose (g/kg)#	–	2.49±1.50	4.47±3.30	—	—	0.352

AMT = amniotic membrane transplantation; IVIG = intravenous immunoglobulin; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis. Data are presented as the number or the mean±standard deviation. Group 1 (S) = conventional steroid (1 mg/kg daily). Group 2 (S plus IVIG) = conventional steroid (1 mg/kg daily) + intravenous immunoglobulin. Group 3 (IVIG) = intravenous immunoglobulin alone. Group 4 (PS) = intravenous steroid pulse therapy (500 mg daily for 3 days). Group 5 (C) = supportive care; (–) = not used.
Boldface values indicate that the variable is statistically significant.
*Kruskal-Wallis test.
†Fisher exact test.
‡Significantly larger proportion than in groups 1 or 5.
§Days from acute ocular complication onset until initial treatment.
‖Data are hydrocortisone equivalent dose.
#Mann–Whitney *U* test.

Table 7. Final Visual Outcome and Chronic Ocular Surface Complication Score According to Treatment

	Group 1 (S)	Group 2 (S plus IVIG)	Group 3 (IVIG)	Group 4 (PS)	Group 5 (C)	*P* Value
Final BCVA (logMAR)	0.44±1.03	0.45±0.77	0.36±0.93	0.32±0.19	0.11±0.23	0.912*/0.751†
COCS	3.31±4.28	3.50±3.71	4.40±3.30	4.00±0.89	2.00±0.89	0.195*/0.067†

BCVA = best-corrected visual acuity; COCS = chronic ocular surface complication score; logMAR = logarithm of the minimal angle of resolution.
Data are mean ± standard deviation. Group 1 (S) = conventional steroid (1 mg/kg daily). Group 2 (S plus IVIG) = conventional steroid (1 mg/kg daily) + intravenous immunoglobulin. Group 3 (IVIG) = intravenous immunoglobulin alone. Group 4 (PS) = intravenous steroid pulse therapy (500 mg daily for 3 days). Group 5 (C) = supportive care.
*Kruskal–Wallis test.
†Nonparametric analysis of covariance.

et al[20] are more reliable because of the higher number of cases and longer follow-up period.[7] Our data also support acute ocular complications as a risk factor of late ocular complications. Compared with Gueudry et al,[20] who used qualitative scales for the evaluation of prognostic factors related to late ocular complications, we used quantitative scoring systems for chronic ocular complications. When we analyzed corneal epithelial erosion or pseudomembrane formation separately, they also turned out to be related significantly to worse final visual outcome and COCS (Table 11, available at www.aaojournal.org). In a personal communication (July 2014), Professor Chie Sotozono considers full-thickness epithelial defect to be more important, rather than diffuse superficial punctate erosion (DSPE) of the acute stage, in relation to visual prognosis of the chronic stage, and includes full epithelial defect only in AOS in Japanese data. However, we found that AOS was still related to chronic ocular complications, although we included both full-thickness epithelial defect and DSPE in AOS in Korean data. The classification of SJS or TEN per se was not associated with final BCVA and COCS. A classification of SJS or TEN was determined according to the degree of body surface involvement, but itself did not seem to predict chronic ocular complications, which corresponded with the results of Yip et al.[7] The patients with high AOS or ASS should be examined more closely to prevent chronic ocular complications.

Interestingly, female gender was the strongest prognostic factor predicting worse final BCVA and chronic ocular surface complications. To our knowledge, there is no report that female gender is related to the severity of SJS or TEN. The fact that drug allergies develop more frequently in women than in men[39] may be associated with this tendency. In addition, among the causative drugs (e.g., NSAIDs, antibiotics, anticonvulsants, antihyperuricemic agents, methazolamide), we looked into the relation of NSAIDs to

Table 8. Subgroup Analysis of the Effect of Variables at the Acute Stage on Chronic Ocular Complications

Variables	Final Best-Corrected Visual Acuity (Logarithm of the Minimum Angle of Resolution)	*P* Value*	Chronic Ocular Surface Complications Score	*P* Value*
Age (yrs)				
≥18 (n = 32 eyes)	0.40±0.90	0.428	3.65±3.50	0.157
<18 (n = 54 eyes)	0.40±0.80		3.13±3.97	
Gender				
Male (n = 38 eyes)	0.09±0.26	**0.002**	1.66±1.58	**<0.001**
Female (n = 48 eyes)	0.65±1.07		4.88±4.21	
SJS/TEN				
SJS (n = 56 eyes)	0.30±0.73	0.311	2.80±2.85	0.092
TEN (n = 30 eyes)	0.60±1.05		4.67±4.64	
AOS				
≥2 (n = 64 eyes)	0.51±0.96	**0.005**	4.22±3.87	**<0.001**
<2 (n = 22 eyes)	0.09±0.30		1.23±1.54	
ASS				
≥8 (n = 44 eyes)	0.54±0.99	0.055	4.59±3.60	**<0.001**
<8 (n = 42 eyes)	0.25±0.67		2.26±3.38	
AMT				
AMT performed (n = 12 eyes)	0.76±1.05	0.104	5.75±4.35	**0.005**
AMT not performed (n = 74 eyes)	0.34±0.82		3.08±0.40	

AMT = amniotic membrane transplantation; AOS = acute ocular involvement score; ASS = acute systemic involvement score; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis.
Data are mean±standard deviation.
Boldface values indicate that the variable is statistically significant.
*Nonparametric analysis of covariance.

Table 10. Prognostic Factors Related to Poor Final Visual Outcome (<20/200) and High Chronic Ocular Complication Score (≥8)

Variables	Poor Final Visual Outcome (Logarithm of the Minimum Angle of Resolution)		High Chronic Ocular Complications Score	
	Unadjusted Odds Ratio (95% Confidence Interval)	*P Value**	*Unadjusted Odds Ratio (95% Confidence Interval)*	*P Value**
Univariate logistic regression				
Age (children/adult)	1.243 (0.949–1.012)	0.731	0.828 (0.192–3.566)	0.800
Gender (female/male)	4.737 (0.971–23.116)	0.055	18.52 (1.003–341.827)	**0.049**
TEN/SJS	3.104 (0.891–10.822)	0.075	2.602 (0.642–10.529)	0.181
AOS (≥2/<2)	1.852 (0.373–9.195)	0.451	7.706 (0.404–147.044)	0.175
ASS (≥8/<8)	2.111 (0.585–7.622)	0.254	3.783 (0.738–19.378)	0.110
AMT (performed/not performed)	4.125 (1.014–16.842)	**0.048**	6.9 (1.532–31.073)	**0.012**
Treatment group†				
2/1	2.086 (0.548–7.945)	0.789	1.327 (0.317–5.544)	0.970
3/1	1.14 (0.145–8.984)		1.142 (0.145–8.984)	
4/1	1.97 (0.22–17.622)		0.555 (0.021–14.526)	
5/1	0.556 (0.021–14.525)		0.555 (0.021–14.526)	
Multivariate logistic regression				
Gender (female/male)	4.975 (0.982–25.203)	0.053	21.564 (1.145–405.977)	**0.040**
AMT (performed/not performed)	4.418 (1.002–23.116)	0.050	8.428 (1.591–44.641)	**0.012**

AMT = amniotic membrane transplantation; AOS = acute ocular involvement score; ASS = acute systemic involvement score; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis.
Boldface values indicate that the variable is statistically significant.
*Univariate logistic regression.
†Group 1 = conventional steroid (1 mg/kg daily). Group 2 = conventional steroid (1 mg/kg daily) + intravenous immunoglobulin. Group 3 = intravenous immunoglobulin alone. Group 4 = intravenous steroid pulse therapy (500 mg daily for 3 days). Group 5 = supportive care.

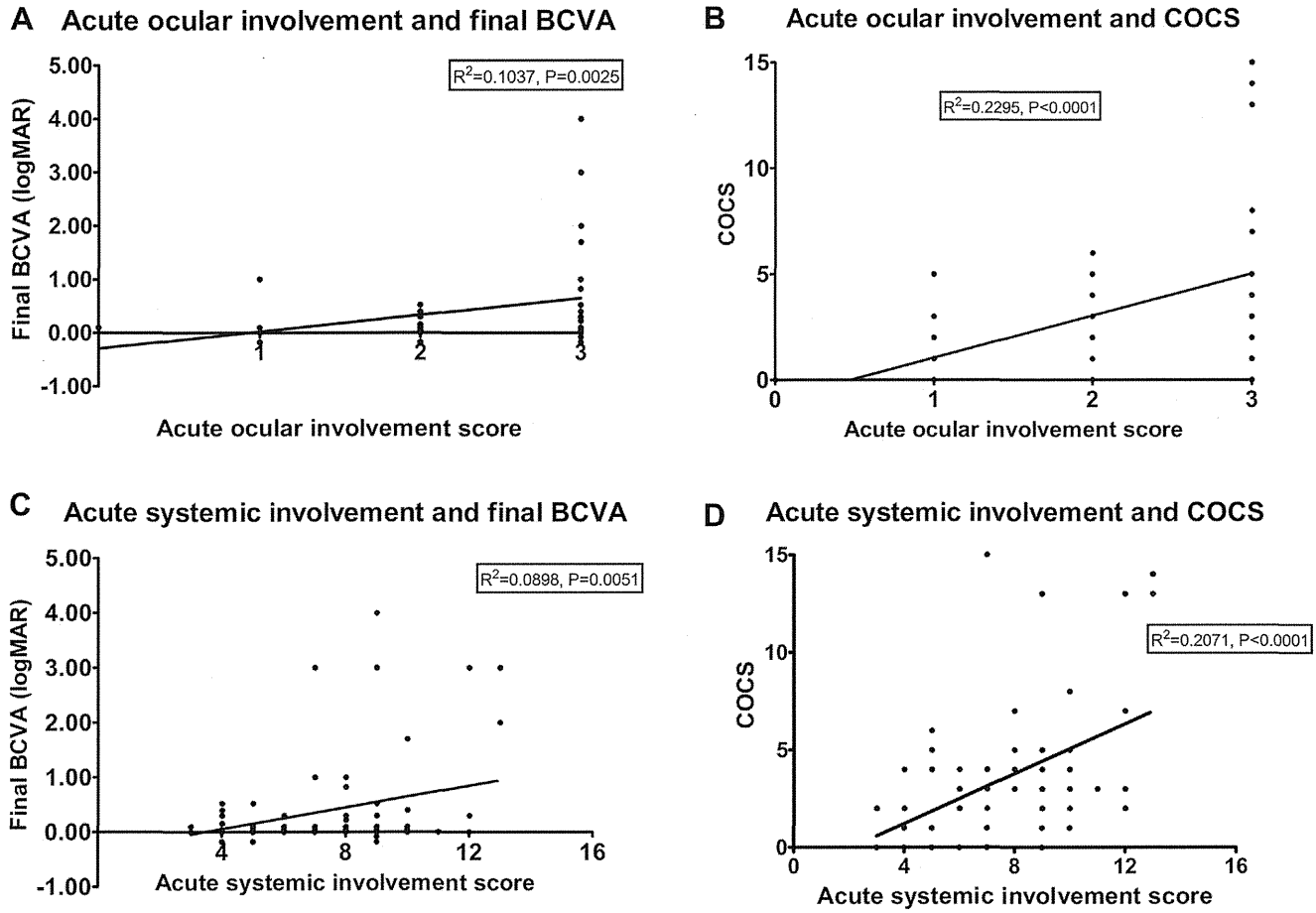

Figure 3. Graphs showing the relationship between acute ocular and systemic involvement with final visual outcome and chronic ocular complications: (**A**) acute ocular involvement score (AOS) versus final best-corrected visual acuity (BCVA); (**B**) AOS versus chronic ocular surface complications score (COCS); (**C**) acute systemic involvement score (ASS) versus final BCVA; and (**D**) ASS versus COCS. logMAR = logarithm of the minimum angle of resolution.

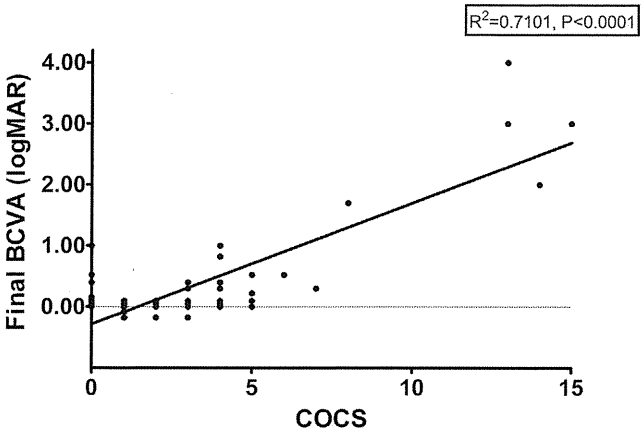

Figure 4. Linear regression analysis between final best-corrected visual acuity (BCVA) and chronic ocular complications score (COCS). logMAR = logarithm of the minimum angle of resolution.

chronic ocular complications, which turned out to be not significant in relationship to chronic ocular complications (Table 12, available at www.aaojournal.org). There are reports that present a significant association between HLA-A*0206 and SJS or TEN with severe ocular surface complications[40] and that demonstrate that NSAID use is a major risk factor for inducing SJS or TEN in patients with HLA-A*0206.[41] Those reports suggest that NSAIDs may be associated with chronic complications in a specific genetic background.[40,41] It is possible that we included patients with various genetic backgrounds, which could have resulted in the lack of a relationship between NSAIDs and chronic complications. Amniotic membrane transplantation at the acute phase of SJS or TEN has been known to be effective in preventing chronic ocular complications.[25,42–46] However, subgroup analysis in patients with an AOS of 3 (Table 9, available at www.aaojournal.org) demonstrated that there were no benefits of AMT in reducing long-term ocular sequelae. Gueudry et al[20] also indicated that AMT was not associated with subsequent ocular complications, which corresponds to our results. Most studies of AMT in SJS or TEN were small case series. In the first case-control study,[42] acute ocular involvements were well controlled, but systemic involvements were not considered. More studies about the effectiveness of AMT in the acute phase of SJS or TEN are required.

Chronic ocular surface complications score showed good correlation with final BCVA. The grading system made by Sotozono et al[9] is an excellent tool for evaluating the severity of chronic ocular involvement in SJS or TEN patients and has been used widely. However, this system, which comprises 13 components and a total of 39 points, is complicated to apply in the clinical setting. Therefore, we simplified the scoring system. This suggests that this simple scoring for chronic ocular complications may enable ophthalmologists to monitor chronic ocular complications in SJS or TEN patients easily and objectively.

The study limitations are its small sample size and retrospective design. The exclusion of those who died also might have introduced bias. Nevertheless, it is meaningful that this study is the first study to compare the effect of several systemic immunomodulatory treatments on final visual outcome and chronic ocular complications in SJS or TEN. Additionally, it is worth noting that important prognostic factors related to chronic ocular complication were revealed and that a simple quantitative scoring system for chronic ocular surface complications was introduced in this study.

In conclusion, there were no therapeutic benefits of early systemic immunomodulatory treatments on chronic ocular complications in SJS or TEN. Female gender and high AOS and ASS were associated with worse chronic ocular complications. As the knowledge of the disease mechanism grows, more research is required to identify novel immunomodulatory treatments to reduce chronic ocular complications.

References

1. Bastuji-Garin S, Rzany B, Stern RS, et al. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. Arch Dermatol 1993;129:92–6.
2. Roujeau J. Stevens-Johnson syndrome and toxic epidermal necrolysis are severity variants of the same disease which differs from erythema multiforme. J Dermatol 1997;24:726–9.
3. Auquier-Dunant A, Mockenhaupt M, Naldi L, et al. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. Arch Dermatol 2002;138:1019–24.
4. Stevens A, Johnson F. A new eruptive fever associated with stomatitis and ophthalmia: report of two cases in children. Arch Pediatr Adolesc Med 1922;24:526–33.
5. Yamane Y, Aihara M, Ikezawa Z. Analysis of Stevens-Johnson syndrome and toxic epidermal necrolysis in Japan from 2000 to 2006. Allergol Int 2007;56:419–25.
6. Mockenhaupt M, Viboud C, Dunant A, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. J Invest Dermatol 2008;128:35–44.
7. Yip L, Thong B, Lim J, et al. Ocular manifestations and complications of Stevens-Johnson syndrome and toxic epidermal necrolysis: an Asian series*. Allergy 2007;62:527–31.
8. Sotozono C, Ueta M, Koizumi N, et al. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. Ophthalmology 2009;116:685–90.
9. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology 2007;114:1294–302.
10. Tugal-Tutkun I, Akova YA, Foster CS. Penetrating keratoplasty in cicatrizing conjunctival diseases. Ophthalmology 1995;102:576–85.
11. Schwartz RA, McDonough PH, Lee BW. Toxic epidermal necrolysis: Part I. Introduction, history, classification, clinical features, systemic manifestations, etiology, and immunopathogenesis. J Am Acad Dermatol 2013;69:173e1–173e13.
12. Chave T, Mortimer N, Sladden M, et al. Toxic epidermal necrolysis: current evidence, practical management and future directions. Br J Dermatol 2005;153:241–53.
13. Halebian PH, Corder VJ, Madden MR, et al. Improved burn center survival of patients with toxic epidermal necrolysis

managed without corticosteroids. Ann Surg 1986;204: 503–12.
14. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. N Engl J Med 1994;331:1272–85.
15. Yang Y, Xu J, Li F, Zhu X. Combination therapy of intravenous immunoglobulin and corticosteroid in the treatment of toxic epidermal necrolysis and Stevens-Johnson syndrome: a retrospective comparative study in China. Int J Dermatol 2009;48:1122–8.
16. Schneck J, Fagot J-P, Sekula P, et al. Effects of treatments on the mortality of Stevens-Johnson syndrome and toxic epidermal necrolysis: a retrospective study on patients included in the prospective EuroSCAR Study. J Am Acad Dermatol 2008;58: 33–40.
17. Kardaun SH, Jonkman MF. Dexamethasone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis. Acta Derm Venereol 2007;87:144–8.
18. Prins C, Vittorio C, Padilla RS, et al. Effect of high-dose intravenous immunoglobulin therapy in Stevens-Johnson syndrome: a retrospective, multicenter study. Dermatology 2003;207:96–9.
19. Campione E, Marulli GC, Carrozzo AM, et al. High-dose intravenous immunoglobulin for severe drug reactions: efficacy in toxic epidermal necrolysis. Acta Derm Venereol 2003;83: 430–2.
20. Gueudry J, Roujeau J-C, Binaghi M, et al. Risk factors for the development of ocular complications of Stevens-Johnson syndrome and toxic epidermal necrolysis. Arch Dermatol 2009;145:157–62.
21. Arstikaitis MJ. Ocular aftermath of Stevens-Johnson syndrome. Arch Ophthalmol 1973;90:376–9.
22. Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctate keratopathy magnitude and its correlation with corneal epithelial permeability. Arch Ophthalmol 2003;121:1537–9.
23. Bastuji-Garin S, Fouchard N, Bertocchi M, et al. SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol 2000;115:149–53.
24. Kim H-I, Kim S-W, Park G-Y, et al. Causes and treatment outcomes of Stevens-Johnson syndrome and toxic epidermal necrolysis in 82 adult patients. Korean J Intern Med 2012;27: 203–10.
25. John T, Foulks GN, John ME, et al. Amniotic membrane in the surgical management of acute toxic epidermal necrolysis. Ophthalmology 2002;109:351–60.
26. Kakourou T, Klontza D, Soteropoulou F, Kattamis C. Corticosteroid treatment of erythema multiforme major (Stevens-Johnson syndrome) in children. Eur J Pediatr 1997;156:90–3.
27. Patterson R, Miller M, Kaplan M, et al. Effectiveness of early therapy with corticosteroids in Stevens-Johnson syndrome: experience with 41 cases and a hypothesis regarding pathogenesis. Ann Allergy 1994;73:27–34.
28. Hirahara K, Kano Y, Sato Y, et al. Methylprednisolone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis: clinical evaluation and analysis of biomarkers. J Am Acad Dermatol 2013;69:496–8.
29. Tseng SC. Acute management of Stevens-Johnson syndrome and toxic epidermal necrolysis to minimize ocular sequelae. Am J Ophthalmol 2009;147:949–51.
30. Viard I, Wehrli P, Bullani R, et al. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. Science 1998;282:490–3.
31. Al-Mutairi N, Arun J, Osama NE, et al. Prospective, non-comparative open study from Kuwait of the role of intravenous immunoglobulin in the treatment of toxic epidermal necrolysis. Int J Dermatol 2004;43:847–51.
32. Trent JT, Kirsner RS, Romanelli P, Kerdel FA. Analysis of intravenous immunoglobulin for the treatment of toxic epidermal necrolysis using SCORTEN: the University of Miami Experience. Arch Dermatol 2003;139:39–43.
33. Brown KM, Silver GM, Halerz M, et al. Toxic epidermal necrolysis: does immunoglobulin make a difference? J Burn Care Rehabil 2004;25:81–8.
34. Huang YC, Li YC, Chen TJ. The efficacy of intravenous immunoglobulin for the treatment of toxic epidermal necrolysis: a systematic review and meta-analysis. Br J Dermatol 2012;167:424–32.
35. Araki Y, Sotozono C, Inatomi T, et al. Successful treatment of Stevens-Johnson syndrome with steroid pulse therapy at disease onset. Am J Ophthalmol 2009;147:1004–11, 11e1.
36. Yip LW, Thong BY, Tan AW, et al. High-dose intravenous immunoglobulin in the treatment of toxic epidermal necrolysis: a study of ocular benefits. Eye (Lond) 2005;19: 846–53.
37. Power WJ, Ghoraishi M, Merayo-Lloves J, et al. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. Ophthalmology 1995;102:1669–76.
38. Kim KH, Park SW, Kim MK, Wee WR. Effect of age and early intervention with a systemic steroid, intravenous immunoglobulin or amniotic membrane transplantation on the ocular outcomes of patients with Stevens-Johnson syndrome. Korean J Ophthalmol 2013;27:331–40.
39. Thong BY, Tan TC. Epidemiology and risk factors for drug allergy. Br J Clin Pharmacol 2011;71:684–700.
40. Ueta M, Sotozono C, Tokunaga K, et al. Strong association between HLA-A*0206 and Stevens-Johnson syndrome in the Japanese. Am J Ophthalmol 2007;143:367–8.
41. 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: 1218–1225.e10.
42. Hsu M, Jayaram A, Verner R, et al. Indications and outcomes of amniotic membrane transplantation in the management of acute Stevens-Johnson syndrome and toxic epidermal necrolysis: a Case-control study. Cornea 2012;31: 1394–402.
43. Gregory DG. Treatment of acute Stevens-Johnson syndrome and toxic epidermal necrolysis using amniotic membrane: a review of 10 consecutive cases. Ophthalmology 2011;118: 908–14.
44. Shammas MC, Lai EC, Sarkar JS, et al. Management of acute Stevens-Johnson syndrome and toxic epidermal necrolysis utilizing amniotic membrane and topical corticosteroids. Am J Ophthalmol 2010;149:203–213e2.
45. Shay E, Kheirkhah A, Liang L, et al. Amniotic membrane transplantation as a new therapy for the acute ocular manifestations of Stevens-Johnson syndrome and toxic epidermal necrolysis. Surv Ophthalmol 2009;54:686–96.
46. Kobayashi A, Yoshita T, Sugiyama K, et al. Amniotic membrane transplantation in acute phase of toxic epidermal necrolysis with severe corneal involvement. Ophthalmology 2006;113:126–32.

Footnotes and Financial Disclosures

Originally received: April 22, 2014.
Final revision: June 24, 2014.
Accepted: August 4, 2014.
Available online: September 25, 2014. Manuscript no. 2014-600.

[1] Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea.

[2] Laboratory of Ocular Regenerative Medicine and Immunology, Seoul Artificial Eye Center, Seoul National University Hospital Clinical Research Institute, Seoul, Korea.

[3] Department of Ophthalmology, Chonnam National University Medical School, Gwangju, Korea.

[4] Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Korea.

[5] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

*Both D.H.K. and K.C.Y. contributed equally as first authors. Both M.K.K. and K.Y.S. contributed equally as corresponding authors.

Financial Disclosure(s):

The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Supported by the Collaborative Research Project of Korea and Japan, Ministry of Science, ICT (Information and Communications Technology) and Future Planning, Republic of Korea (grant no.: 2012K2A2A4020148).

Abbreviations and Acronyms:
AMT = amniotic membrane transplantation; **AOS** = acute ocular involvement score; **ASS** = acute systemic involvement score; **BCVA** = best-corrected visual acuity; **C** = supportive care; **COCS** = chronic ocular surface complications score; **IVIG** = intravenous immunoglobulin; **logMAR** = logarithm of the minimum angle of resolution; **NSAID** = nonsteroidal anti-inflammatory drugs; **OR** = odds ratio; **PS** = systemic pulse steroid; **S** = systemic steroids; **SJS** = Stevens-Johnson syndrome; **TEN** = toxic epidermal necrolysis.

Correspondence:
Mee Kum Kim, MD, PhD, Department of Ophthalmology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Korea. and Kyoung Yul Seo, MD, Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Korea. E-mail: kmk9@snu.ac.kr

Kyoung Yul Seo, MD, Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Korea. E-mail: SEOKY@yuhs.ac.

Progress in Retinal and Eye Research xxx (2015) 1–21

Contents lists available at ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

Review

Ocular surface reconstruction using stem cell and tissue engineering

Takahiro Nakamura [a, *], Tsutomu Inatomi [b], Chie Sotozono [b], Noriko Koizumi [b], Shigeru Kinoshita [a]

[a] Department of Frontier Medical Sciences and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[b] Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form
8 July 2015
Accepted 8 July 2015
Available online xxx

Keywords:
Ocular surface
Stem cell
Tissue engineering
Growth factor
Severe ocular surface disease
Regenerative medicine

ABSTRACT

Most human sensory information is gained through eyesight, and integrity of the ocular surface, including cornea and conjunctiva, is known to be indispensable for good vision. It is believed that severe damage to corneal epithelial stem cells results in devastating ocular surface disease, and many researchers and scientists have tried to reconstruct the ocular surface using medical and surgical approaches. Ocular surface reconstruction via regenerative therapy is a newly developed medical field that promises to be the next generation of therapeutic modalities, based on the use of tissue-specific stem cells to generate biological substitutes and improve tissue functions. The accomplishment of these objectives depends on three key factors: stem cells, which have highly proliferative capacities and longevities; the substrates determining the environmental niche; and growth factors that support them appropriately. This manuscript describes the diligent development of ocular surface reconstruction using tissue engineering techniques, both past and present, and discusses and validates their future use for regenerative therapy in this field.

Contents

* Corresponding author. Department of Frontier Medical Sciences and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto 602-0841, Japan.
E-mail address: tnakamur@koto.kpu-m.ac.jp (T. Nakamura).

http://dx.doi.org/10.1016/j.preteyeres.2015.07.003
1350-9462/

1. Introduction

The concept of an "ocular surface" is widely recognized in the field of ophthalmology, and our understanding of the role of ocular surface biology and immunology has been greatly improved by the numerous research studies carried out in this field (Thoft and Friend, 1979). Although the normal ocular surface comprises only 1/6 of the outer wall of the eye, it supports several of the eye's major functions, as it is covered with highly specialized corneal and conjunctival epithelia, formed by two phenotypically different types of epithelial cell. Over the past thirty years, several scientific discoveries such as the identification of corneal epithelial stem cells, the establishment of novel methods in epithelial culturing and the understanding of extracellular matrices and growth factors have enabled a novel surgical approach to treatment of ocular surface disorders, using regenerative medicine.

Based on tissue engineering, regenerative medicine is a newly developed area that uses somatic stem cells to generate biological substitutes and improve tissue functions (Langer and Vacanti, 1993). Success depends on three key factors: stem cells, extracellular matrices and growth factors. A variety of trials are currently in development, based on the utilization of stem cells and appropriate substrates to produce substitutes capable of reconstructing damaged and diseased tissues. In the field of ophthalmology, the production of tissue organs *in vitro* shows great promise, especially with regard to the anterior segment of the eye (Pellegrini et al., 1997).

Severe ocular surface disease (OSD) due to thermal and chemical burns, Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP) or other conditions currently poses a serious clinical challenge for ophthalmologists worldwide. In these cases, the corneal epithelial stem cells located in the corneal limbus are destroyed, and coverage of the corneal surface by invading neighboring conjunctival epithelial cells results in neovascularization, chronic inflammation, ingrowth of fibrous tissue and stromal scarring. This severely compromises the ocular surface and seriously diminishes visual acuity (Chiou et al., 1998; Kinoshita et al., 2001; Tseng, 1989). Conventional treatment methods have generally proved unsatisfactory, and the long-term consequences of these ocular disorders are devastating. Clinically useful and effective surgical techniques for ocular surface reconstruction (OSR) are therefore needed for such patients.

In this present paper, we describe the history, recent advances, current developments and future challenges relating to OSR in both its basic science and clinical aspects, as well as providing novel clinical information for the treatment of severe OSD.

2. Ocular surface reconstruction

The concept of OSR is widely accepted in the field of ophthalmology, and our understanding of the role of the ocular surface has been greatly improved by numerous research studies. Various surgical procedures have been developed over the past 30 years to treat and reconstruct severely damaged or diseased ocular surface epithelia.

2.1. Conjunctival transplantation, keratoepithelioplasty and limbal transplantation

The concept of OSR was first reported in relation to an autologous conjunctival transplantation for unilateral chemical injury in Thoft's description of conjunctival tissue transplantation for unilaterally affected chemical injuries (Thoft, 1977). The surgery was performed by removing pathological scarred tissue from a patient's corneal surface and placing four pieces of conjunctival autograft taken from the contralateral eye at the limbus in order to reconstruct the cornea by regenerating conjunctival epithelial cells from these autografts. Subsequently, Thoft described the similar surgical technique of keratoepithelioplasty (Thoft, 1984), which employed a different tissue source (donor corneal lenticules) to regenerate corneal epithelial cells. Although the concept of corneal epithelial stem cells was not established at that time, the cell-level biological differences between regenerated corneal and conjunctival epithelia were known. Over time, keratoepithelioplasty has gradually gained acceptance despite initial disputes among researchers because it is a form of epithelial allograft (Kaufman, 1984). In fact, keratoepithelioplasty has proved to be dramatically effective in treating peripheral corneal ulcers, including Mooren's ulcer (Kinoshita et al., 1991), supplying both a regenerated corneal epithelium and an appropriate corneal substrate for inhibiting conjunctival invasion onto the cornea. Sun's group proposed the corneal limbal stem cell concept (Schermer et al., 1986), which had a tremendous impact on the development of keratoepithelioplasty, leading to autologous limbal transplantation (LT) (Kenyon and Tseng, 1989). Tsai and Tseng then introduced allogenic LT, aimed at achieving a permanent lifespan for regenerated corneal epithelium by means of stem cell transplantation, although intensive immunosuppressive therapy was also needed (Tsai and Tseng, 1994). These surgical procedures are classed as "cellular surgery"—a form of primitive regenerative

Please cite this article in press as: Nakamura, T., et al., Ocular surface reconstruction using stem cell and tissue engineering, Progress in Retinal and Eye Research (2015), http://dx.doi.org/10.1016/j.preteyeres.2015.07.003

medicine—as they are a form of *in vivo* expansion of corneal epithelial cells. Significantly, Kim and Tseng subsequently reported that amniotic membrane (AM) transplantation was capable of inhibiting pathological subepithelial scarring in OSR (Kim and Tseng, 1995). Since that time, AM transplantation combined with limbal allografts has been used to treat certain challenging occurrences of severe OSD (Tsubota et al., 1996).

2.2. Cultivated limbal epithelial transplantation (CLET)

While corneal epithelial transplantations (including keratoepithelioplasty and LT) have indisputably contributed to improved clinical outcomes for OSR in a range of clinical situations, an autologous LT needs quite a large section of limbal tissue from the healthy eye and cannot be applied if the disease is bilateral. In order to improve the surgical results of OSR for severe OSD, CLET needs to be developed *in vitro* from a small portion of limbal epithelium (Fig. 1).

2.2.1. The history of creating corneal epithelial sheets

In the initial stages of OSR during the 1980s, at least two investigative approaches were adopted in attempting to create an epithelial sheet. One was the direct sampling of corneal epithelial sheets, using dispase and gentle mechanical treatment. In rabbit experiments, the corneal epithelial sheet, taken directly from the *in vivo* cornea, attached itself to the corneal stroma but peeled away easily during lid movement, making it quite difficult to preserve the corneal surfaces (Gipson and Grill, 1982).

A second approach was to develop a cultivated epithelial cell sheet. In dermatological research, Green successfully established a procedure for cultivating epidermal cell sheets (Rheinwald and Green, 1975), focusing particular attention on the tissue-engineered cultivated corneal epithelial cell sheet. The cultivation of corneal epithelial cells on the scraped rabbit corneal stroma was examined by Friend et al. (1982), and several other substrates, such as collagen matrix and hydrogel coated with fibronectin, were also investigated in developing this procedure (Kobayashi and Ikada, 1991; Minami et al., 1993). However, until 1997, there were no clinical reports regarding the success of OSR using the cultivated corneal epithelial cell sheets, probably because of the poor understanding of surrounding corneal epithelial stem cell cultivation and/or its proper substrate. Some groups tried to reconstruct all three layers of corneal tissue—a "corneal equivalent"—using cell-lines arranged by natural and synthetic polymers (Griffith et al., 1999). This kind of corneal equivalent is now available for use in testing experimental drug toxicity and efficacy but not for clinical use (because of the use of immortalized cell lines).

2.2.2. Development of CLET

From the mid-1990s, attention has focused on the development of regenerative corneal epithelial cell therapy using tissue-engineered techniques as a new approach to OSR. The first successful OSR procedure using autologous CLET for patients with unilateral OSD was reported by Pellegrini et al. (Pellegrini et al., 1997). They developed a surgical method to reconstruct stratified corneal epithelial cell sheets on petrolatum gauze or a soft contact lens as carrier, treating two patients. It seems likely that their success can be accounted for by their adoption of a well-recognized epidermal keratinocyte-culturing method, including the use of mouse-derived 3T3 feeder layers to maintain epithelial stem cells. Since then, scientists worldwide have sought to devise novel and better methods for OSR. Again, in considering reconstruction of the ocular surface, the three key elements are the cell source and its proper substrate and growth factors. Following establishment of a suitable substrate, many researchers investigated the use of AM, fibrin and a temperature-responsive culture dish as carrier

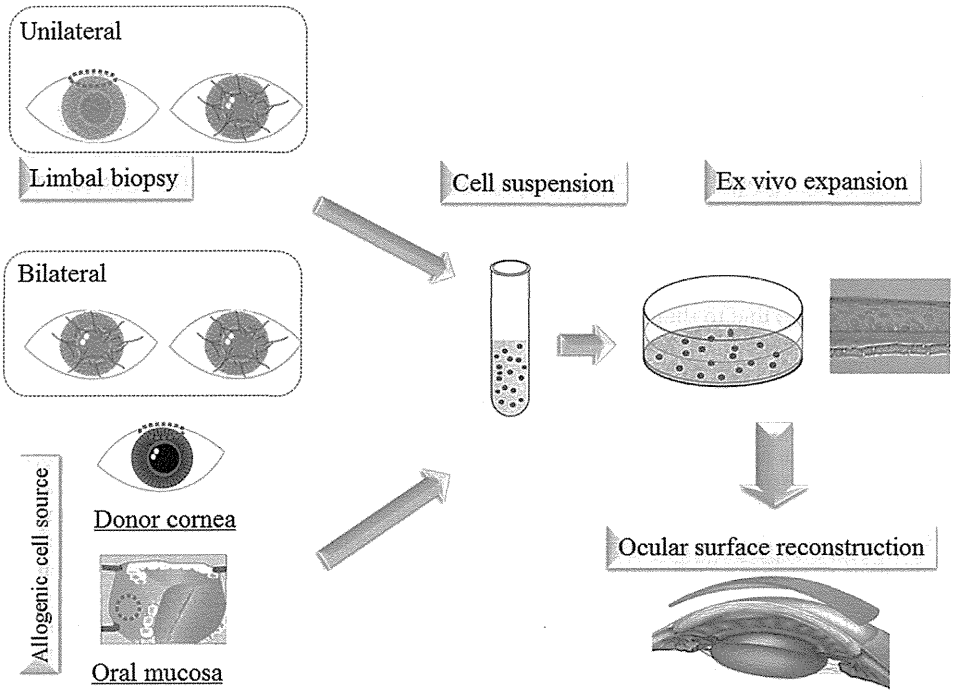

Fig. 1. Conceptual diagram of OSR via regenerative therapy. For patients with unilateral severe OSD, autologous corneal limbal epithelial cells from a biopsy of the uninjured eye (dashed circle) are used for cell culturing. For patients with bilateral severe OSD, allogenic cell sources (donor cornea or autologous oral mucosa) are used for cell culturing. The resultant cell suspensions were seeded onto the proper substrate under appropriate *in vitro* conditions. Finally, we successfully tissue-engineered the cultured sheet and transplanted it to the diseased corneal surface.

Please cite this article in press as: Nakamura, T., et al., Ocular surface reconstruction using stem cell and tissue engineering, Progress in Retinal and Eye Research (2015), http://dx.doi.org/10.1016/j.preteyeres.2015.07.003

(Koizumi et al., 2000a; Nishida et al., 2004a; Rama et al., 2001; Tsai et al., 2000). From a clinical perspective, AM can serve not only as a proper epithelial carrier but also as a healthy substrate to cover a damaged ocular surface; the success of CLET using AM has been reported by a majority of groups worldwide (Baylis et al., 2011; Zhao and Ma, 2015). In addition, Sangwan has recently reported a novel surgical technique that combines AM transplantation and cultivation of small limbal explant with fibrin glue (Sangwan et al., 2012).

2.2.3. Clinical outcomes of autologous CLET

The ideal therapy for unilateral severe OSD—as caused, for instance, by thermal and chemical burns—is the use of autologous cultivated limbal epithelial sheets, with harvesting of much smaller pieces of limbal biopsy to prevent damage to the healthy or uninjured eye. With this in mind, several researchers have reported the successful use of autologous CLET for OSR (Grueterich et al., 2002; Nakamura et al., 2004b; Sangwan et al., 2003; Schwab et al., 2000; Tsai et al., 2000). These initial reports offer hope to patients with unilateral lesions or severe ocular surface damage. Long-term clinical assessment of autologous CLET was reported by Rama (Rama et al., 2010), confirming that autologous cultivated limbal stem cells represent an adequate long-term source for tissue-engineered transplants; clinical results reported success at up to 10 years in more than 75% of the patients treated. Importantly, this demonstrates the relationship between clinical results and the percentage of p63 (+) cells in cultivated graft. (Cultures in which p63-bright cells accounted for more than 3% of the total number of clonogenic cells were associated with successful transplantation in 78% of patients. In contrast, cultures in which such cells made up 3% or less of the total number of cells were associated with successful transplantation in only 11% of patients). In our long-term clinical cases (mean period 48 months; longest follow-up period 80 months), autologous CLET successfully reconstructed the corneal surface, but all cases showed varying degrees of mild superficial conjunctivalization extending from the limbal region (Fig. 2). These long-term clinical results strongly support the conclusion that tissue-engineered autologous CLET can be useful for reconstructing the ocular surface in cases of unilateral severe OSD. We further posit that the cultivation of limbal epithelial cells harvested from much smaller specimens is possible but difficult to achieve using conventional culturing techniques; these must be further developed to produce a sufficiently stratified epithelium that will hopefully include limbal stem cells.

2.2.4. Clinical outcomes of allogenic CLET

Although autologous CLET is the safest and most reliable procedure, bilaterally affected severe OSD cannot be treated by this means. In order to treat these bilateral cases, we have developed allogeneic CLET, using AM as a culture substrate (Kinoshita et al., 2004; Koizumi et al., 2001a). While acute-phase patients with persistent epithelial defects received allogeneic CLET for the

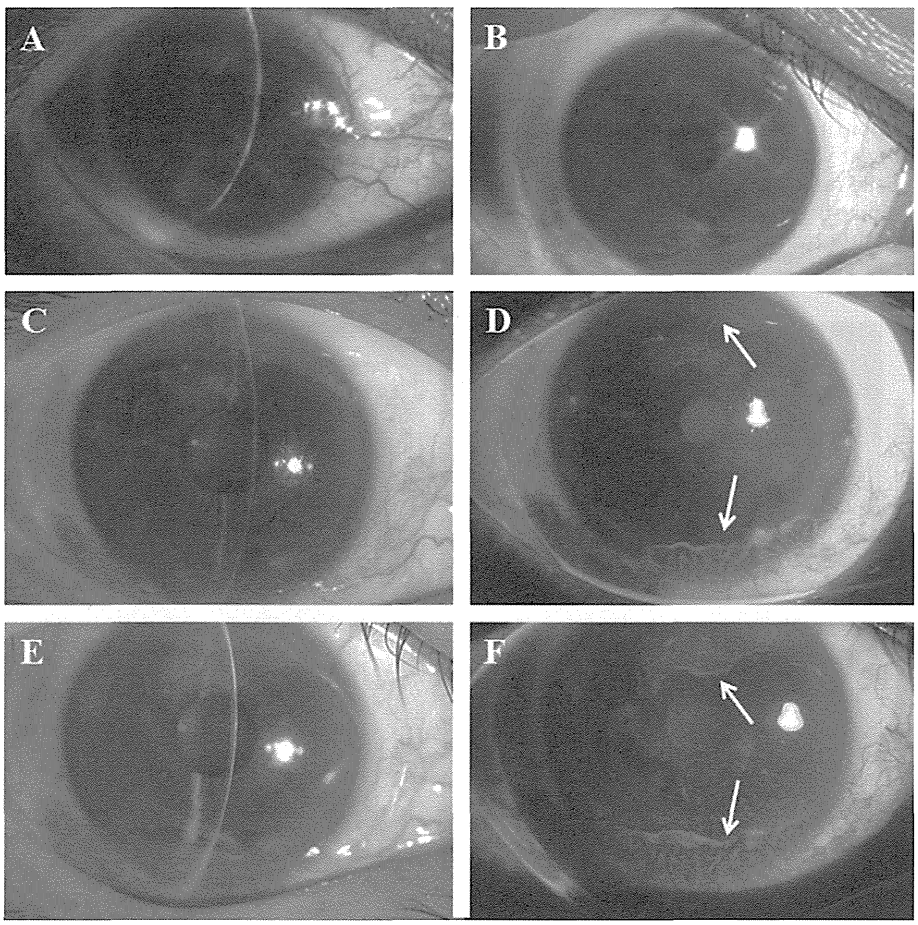

Fig. 2. Representative long-term clinical results of autologous CLET in patient with chemical injury. (A) Before transplantation, the eye manifested severe destruction of the ocular surface, with conjunctivalization and neovascularization. Postoperative appearance at 1 year (B), 5 years (C, D) and 7 years (E, F) shows a relatively smooth, epithelialized corneal surface with minimal corneal scarring and inflammation. During long-term follow-up, varying degrees of mild peripheral conjunctivalization were observed (D, F; white arrows).

Please cite this article in press as: Nakamura, T., et al., Ocular surface reconstruction using stem cell and tissue engineering, Progress in Retinal and Eye Research (2015), http://dx.doi.org/10.1016/j.preteyeres.2015.07.003

purpose of covering the corneal surface and reducing ocular surface inflammation, chronic-phase patients received CLET to improve visual acuity. We have transplanted allogeneic cultivated corneal limbal epithelial cells in 39 eyes of 36 patients with severe OSD, including acute and chronic phases of SJS, OCP and thermal and chemical injuries (Fig. 3). During the postoperative 1–3 years, most of the transplanted sheets successfully survived on the ocular surfaces and maintained their transparency with the aid of immunosuppressive treatments.

It is worth noting that, in acute-phase patients, the severe preoperative ocular surface inflammation that had not been controlled by conventional treatments decreased rapidly post transplantation (Koizumi et al., 2001b); in chronic-phase patients, long-term visual outcome and epithelial stability varied. In a case of severe chemical injury, the transplanted corneal epithelial sheet was found to be transparent and stable as much as 10 years after transplantation, with only minimal conjunctival inflammation during the follow-up period. On the other hand, in SJS patients, mild to moderate ocular surface inflammation and subsequent rejection occurred after allogeneic CLET. While subconjunctival fibrosis had not progressed in SJS, conjunctival scarring (symblepharon and shortening of the conjunctival fornix) had progressed in OCP. Our clinical observations confirmed that the phenotypes of transplanted cells gradually changed from donor to host epithelial cells over a couple of years, but subepithelial scarring and neovascularization did not progress. This phenomenon can be explained as a mild rejection of the transplanted corneal epithelial cells. For that reason, we strongly believe that postoperative management, especially immunosuppressive therapy, is critical in ensuring survival of the transplanted graft. Although graft survival was brief in some chronic cases, the ocular surface maintained its transparency and patients gained better visual function in comparison to their condition before surgery. Importantly, it was noted that the rejected cultivated transplants were easily removed from patients' ocular surfaces at the time of second surgery, and the exposed corneal stroma were found to be fairly transparent, with fewer scarring changes (Nakamura et al., 2003c).

2.2.5. Phenotypic investigation of allogenic CLET

Although our clinical assessments of allogenic CLET yielded favorable outcomes from the perspective of corneal stabilization, long-term phenotypic analyses of allogenic corneal epithelial transplants to the ocular surface would be required to confirm the direct evidence of graft survival. To this end, we compared our clinical observations with the results of long-term cell biological phenotype analysis of allogeneic CLET (Nakamura et al., 2010) (Fig. 4). In that report, we noted that, in clinical conjunctival phenotypic grafts, the transplanted cells were gradually replaced by surrounding conjunctival epithelial cells. In contrast, clinical corneal phenotypic grafts demonstrated that transplanted cells could indeed survive for a long period of time. Those findings have valuable clinical implications and provide novel information on allogeneic CLET, suggesting reasons for the phenotypic diversities of these grafts.

2.3. Cultivated oral mucosal epithelial transplantation (COMET)

As severe OSD is mostly bilateral, ophthalmologists worldwide have no choice but to select allogeneic LT or CLET, resulting in intensive and prolonged postoperative immunosuppressive therapy. These drawbacks have led us to examine whether the ocular surface could be reconstructed by using an autologous mucosal epithelium of non-ocular surface origin. In so doing, we (and other groups) have developed COMET as a substitute for corneal epithelial cells (Nakamura et al., 2003a, 2004a; Nakamura and Kinoshita, 2003; Nishida et al., 2004b).

2.3.1. Development of COMET

In the past, several groups have investigated the possibility of using oral mucosa for OSR. Ballen used oral mucosal grafts that included both epithelial and subepithelial tissues in both human and rabbit eyes, finding that they vascularized heavily with early fibrosis (Ballen, 1963). Gipson et al. transplanted oral mucosal

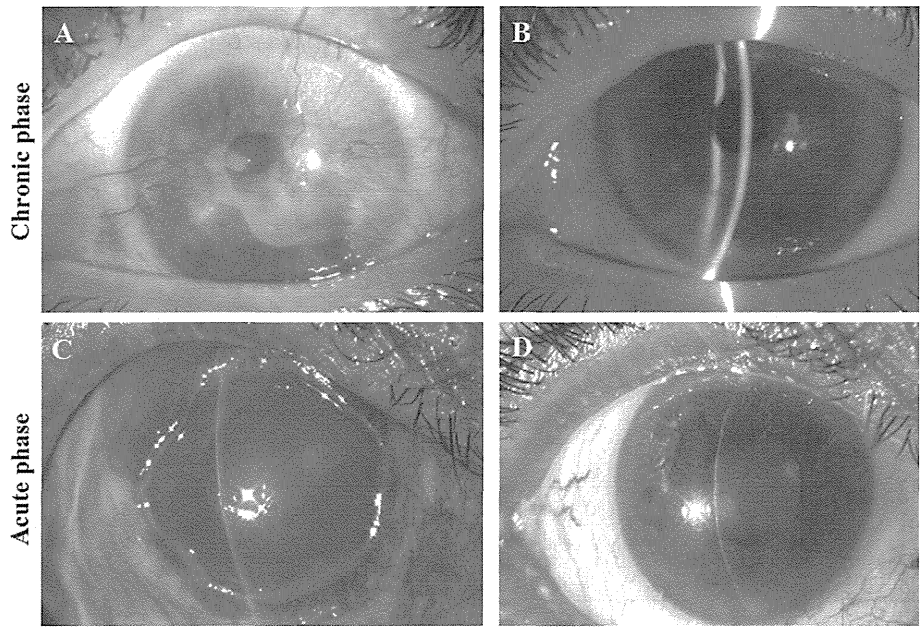

Fig. 3. Representative clinical results of allogenic CLET in patient with chronic (A, B) and acute (C, D) phase of chemical injury. Before transplantation, the eyes manifested conjunctivalization with corneal scarring (A) and persistent epithelial defects surrounded by inflammatory subconjunctival fibrosis (C). At 6 years (B) and 2 years (D) post-transplantation, the ocular surface was stable, without epithelial defects. Modified with permission from Kinoshita et al. (2004).

Please cite this article in press as: Nakamura, T., et al., Ocular surface reconstruction using stem cell and tissue engineering, Progress in Retinal and Eye Research (2015), http://dx.doi.org/10.1016/j.preteyeres.2015.07.003

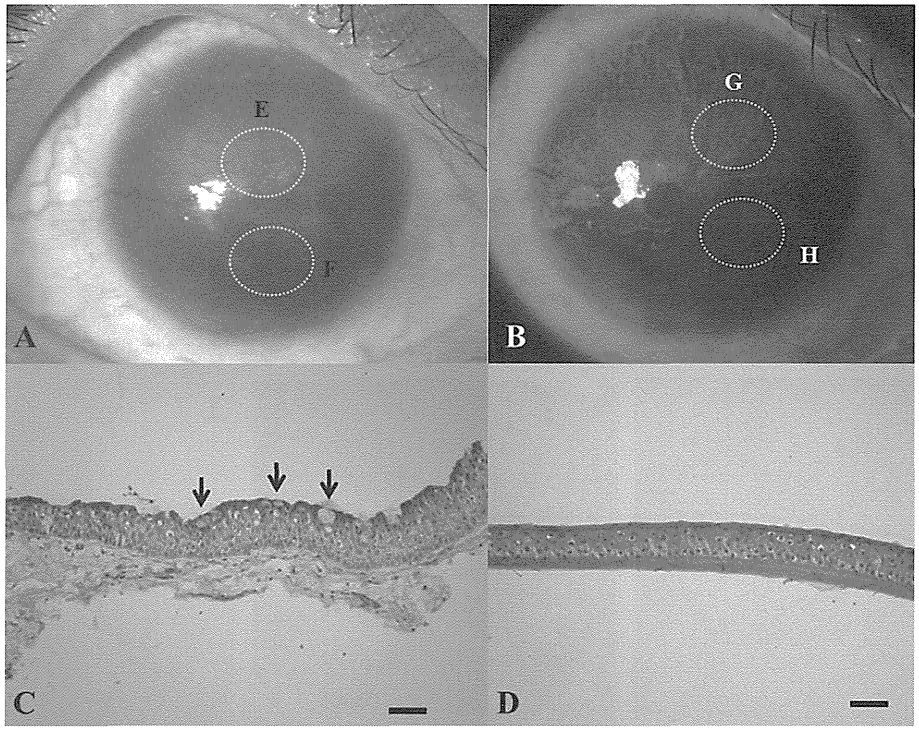

Fig. 4. Representative slit-lamp photographs after allogenic CLET in patient with SJS. Slit-lamp examination revealed that the upper part of the corneal surface was apparently covered with conjunctiva (A; white circle E). However, slit-lamp examination revealed that the lower-nasal part of the corneal surface was still transparent (A; white circle F). Fluorescein staining showed a comparatively clear demarcation between corneal and conjunctival phenotypes (B). The corneal phenotype area showed comparatively smooth epithelium with no fluorescein staining (B; white circle H), but the conjunctival phenotype area revealed light and stippled staining with fluorescein. (B; white circle G). The cross-section of white circle E disclosed 5 to 6 stratified layers of conjunctival-like epithelial cells and also included goblet-cell-like cells (black arrows) (C). Cross-section of white circle F showed 5 to 6 stratified cell layers and cornea-like epithelial cells (D). Scale bars: C, D = 50 μm. Modified with permission from Nakamura et al. (2010).

epithelial cells freed by Dispase II treatment of underlying connective tissue onto the rabbit ocular surface (Gipson et al., 1986). They reported that it was feasible to transplant *in vivo* oral mucosal epithelium to corneal-limbal areas but that it was not maintained in central corneal regions. By general consensus, the character of epithelial cell is thought to depend on the underlying substrate, and AM is a good substrate for cultivating mucosal epithelium (Fig. 5). Based on these considerations, we first propose that oral mucosal epithelial cells cultivated on AM may be able to differentiate into cornea-like epithelial cells under cell culture conditions (Nakamura et al., 2003a; Nakamura and Kinoshita, 2003). After intensive investigations, we successfully generated a well-stratified and differentiated rabbit and human cultivated oral mucosal epithelial sheet that appeared very similar to normal *in vivo* corneal epithelium, successfully performing autologous transplantation of these cells onto rabbit corneas (Fig. 5). These results demonstrate that human cultivated oral mucosal epithelial sheets can function as an ocular surface epithelium and that COMET is a feasible method of OSR.

2.3.2. Clinical outcomes of autologous COMET

In light of these experimental results, this method was initially applied to six eyes of four patients with severe OSD, and the ocular surfaces were successfully reconstructed (Nakamura et al., 2004a) (Fig. 6). During follow-up (13.8 ± 2.9 months), visual acuity showed improvement in all eyes, and the ocular surface remained stable. Although all eyes showed peripheral neovascularization with epithelial thickening, use of this novel tissue-engineering technique generated from autologous oral mucosa avoids the need to administer intensive and prolonged immunosuppressive therapy, so reducing the risk of postoperative complications.

We reported long-term clinical data on 19 eyes that received COMET (mean follow-up period 55 months; longest follow-up period 90 months) (Nakamura et al., 2011) (Fig. 6). The study included 19 eyes of 17 patients in the chronic phase of severe OSD; clinical results were evaluated and graded on a scale according to severity (Sotozono et al., 2007). Clinical safety was evaluated in cases of persistent epithelial defect (PED), ocular hypertension and infections. During follow-up, best-corrected visual acuity was improved in 18 eyes (95%), and visual acuity at the postoperative 36th month was improved in 10 eyes (53%). During the follow-up period, clinical conjunctivalization was significantly inhibited, and corneal opacification tended to improve. All eyes showed various degrees of superficial corneal vascularization, but this gradually reduced, and its activity was comparatively stable from 6 months after surgery. Symblepharon formation was also significantly inhibited; 7 of the 19 eyes showed PED at least once during the long-term follow-up. Ocular hypertension was observed in a total of 3 eyes. Corneal infection was observed mainly within 6 months after transplantation, and methicillin-resistant staphylococcus aureus was found to be the only cause of infection. Most recently, in order to clarify the effectiveness, disease-specific results and safety of COMET, all of the clinical data of all 72 patients treated with COMET since 2002 were analyzed (Sotozono et al., 2013). The findings of this retrospective study confirmed that long-term visual improvement can be obtained in end-stage severe OSDs, and that COMET offered substantial visual improvement even for patients with severe tear deficiency. The findings also showed that patients with corneal blindness resulting from severe OSDs such as SJS benefited from critical improvement of visual acuity. It was concluded that COMET is a safe and effective treatment for improvement of the

Please cite this article in press as: Nakamura, T., et al., Ocular surface reconstruction using stem cell and tissue engineering, Progress in Retinal and Eye Research (2015), http://dx.doi.org/10.1016/j.preteyeres.2015.07.003