Table 1. Backgrounds and HLA-B diplotypes of Japanese carbamazepine-related SJS/TEN patients

ID[a]	ADR type	Sex/Age	Severity score in ophthalmic disorders	Highest BT (°C)	Total area of blistering skin (%)	Systemic complications	Result of DLST to CBZ	Period of onset for CBZ (days)	Coadministered drugs		HLA-B diplotype	
									Drug name	DLST result/period of onset	High resolution	Low resolution
1 (1)	TEN	M/73	1	>39	20	Neutropenia	–	14	Potassium citrate/sodium citrate hydrate	–/4 days	**1511**/4801	**B75**/B48
						Liver dysfunction			Allopurinol	–/>5 years		
									Etizolam	–/>5 years		
									Sodium pravastatin	–/>5 years		
2 (5)[b]	SJS	F/6	At least 1[c]	>37.0	<10%	GI tract disturbance	Not tested	9	None		4006/5101	B61/B51
3 (6)[b]	SJS	F/52	At least 1[c]	Unknown	<10%	Neutropenia Liver dysfunction	Not tested	14	Zonisamide	Not tested/346 days	4601/5901	B46/B59
4	SJS	M/52	0	38	1	GI tract disturbance Neutropenia Liver dysfunction Renal dysfunction	Not tested	51	Tegafur/gimeracil/oteracil potassium	Not tested/38 days	0702/5201	B7/B52
5	SJS	M/32	1	39	5	Liver dysfunction	Not tested	42	None		4002/5401	B60/B54
6 (2)	SJS	F/42	3	>39	5	GI tract disturbance	–	Shorter than 34	Sodium diclofenac	–/1 year	4001/5201	B60/B52
									L-carbocysteine	–/1 year		
									Cefteram pivoxil	–/4 days		
									Olopatadine hydrochloride	Not tested/unknown		
7	SJS	F/64	At least 1[c]	>37.0	10	Liver dysfunction	+	13	Mecobalamin	Not tested/13 days	**1511**/4002	**B75**/B60
8 (3)	SJS	M/45	3	>37.0	5	Liver dysfunction	Not tested	49	None		4801/5601	B48/B56
9 (4)	SJS	M/54	0	<37.0	0.5	None	+	34	None		1501/3501	B62/B35
10	TEN	M/38	3	40.3	40	Liver dysfunction	+	15	Troxipide	–/8 days	1302/4403	B13/B44
									Levofloxacin hydrate	–/15 days		
									Mecobalamin	–/9 days		
									Acyclovir	–/9 days		
11 (7)	TEN	M/17	3	39.7	20	Respiratory involvement Neutropenia Liver dysfunction	+	5	Zonisamide	+/33 days	4601/5601	B46/B56
									Amoxicillin hydrate	+/1 day		
									Promethazine methylenedisalicylate	Not tested/1 day		
12[d]	SJS	M/6	1	Unknown	<10%	Liver dysfunction	–	145	Zonisamide	+/24 days	**1511**/4006	**B75**/B61
13	Probable SJS	F/54	Unknown	<37.0	>10%	Liver dysfunction	Not tested	22	Sodium pravastatin	Not tested/unknown	4006/4403	B61/B44
									Nifedipine	Not tested/81 days		
									Etizolam	Not tested/15 days		
									Lansoprazole	Not tested/46 days		
									Sodium risedronate hydrate	Not tested/46 days		
14	Probable SJS	F/36	At least 1[c]	Unknown	5	None	+	15	Timiperone	Not tested/1 day	1301/**1511**	B13/**B75**
15	Atypical SJS	F/65	1	37.4	0.1	None	+	9	None		**1511**/3501	**B75**/B35

BT, body temperature; DLST, drug lymphocyte stimulation test; CBZ, carbamazepine.
[a]Number in parentheses is ID # from our previous study (Kaniwa et al., 2008).
[b]These patients were also included in Ikeda et al. (2010)
[c]Ophthalmic complications were observed, but severity was unknown.
[d]This patient was excluded from statistical analyses due to likely zonisamide-induced SJS.

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Table 2. Population allele frequencies of individual types of HLA-B75 in various ethnic groups

Ethnic group	Population allele frequencies reported in allelefrequencies.net website[a]				
	*HLA-B*1502*	*HLA-B*1515*	*HLA-B*1521*	*HLA-B*1508*	*HLA-B*1511*
Japanese	0.001	Data unavailable	Data unavailable	Data unavailable	**0.004–0.008**[b,c]
Koreans	0.002	0.000	0.000	0.000	0.020
Han Chinese	**0.019–0.124**[b]	0.010	0.000–0.002	0.005–0.015	0.000–0.017[d]
Thai	**0.061–0.085**[b]	Data unavailable	**0.007–0.010**[b]	0.010	**0.010**[b]
Indians	**0.000–0.060**[b]	Data unavailable	Data unavailable	**0.005–0.033**[b]	Data unavailable
Caucasians (West Europe)	0.000	0.000	0.000	0.000–0.004	0.000–0.003
Caucasians (East Europe)	0.000	0.000	0.000	0.000–0.009	0.000
Sub-Saharan Africans	0.000	0.000–0.008	Data unavailable	0.000	0.000
Hispanics	0.000	0.004–0.008	0.000	0.000–0.006	0.000
Arabians	0.000	0.000	0.000	0.000–0.007	0.000
Australian aborigine	0.000–0.007	Data unavailable	0.026–0.135	Data unavailable	Data unavailable

[a]New Allele Frequency Database: http://www.allelefrequencies.net/ (Middleton et al., 2003).
[b]SJS/TEN patients carrying the allele shown in the second row have been reported in the study using an ethnic group shown in the first column.
[c]The frequency of 0.1 was reported by Tanaka et al. (1996).
[d]Higher value than 0.038 in Han Chinese in Beijing was recently reported by Yang et al. (2010).

factor for carbamazepine-induced SJS/TEN for Thai and Australian aborigine. Interestingly, HLA-B75 has not been detected in carbamazepine-induced SJS/TEN Caucasian patients (Lonjou et al., 2006). This may be due to extremely low allele frequencies or no existence of HLA-B75 subfamilies.

*HLA-B*1502* has been reported to have associations with SJS/TEN caused by other aromatic antiepileptic drugs such as phenytoin and lamotrigine in Han Chinese and Thai (Man et al., 2007; Locharernkul et al., 2008). In this study we detected a patient carrying *HLA-B*1511* whose causative drug was probably zonisamide, an aromatic antiepileptic drug. Therefore, *HLA-B*1511* may be also involved in the onset of SJS/TEN induced by other aromatic antiepileptic drugs as well as *HLA-B*1502*, although further investigation is needed.

The odds ratio of *HLA-B*1511* for SJS/TEN obtained in this study was low in comparison with those observed in Thai, Indians, and Han Chinese in Taiwan (25.5, 71.4, and 25.04 respectively) (Chung et al., 2004; Locharernkul et al., 2008; Mehta et al., 2009). One reason for this may be the low allele frequency (<0.01) of *HLA-B*1511* among the Japanese. The administration of multiple drugs to Japanese patients may also contribute to the low odds ratio. Indeed, on average, more than three drugs were administered to the patients in this study. We concluded that patients receiving multiple drugs developed SJS/TEN due to carbamazepine by comparing the periods of latency of the individual drugs prior to SJS/TEN onset. However, we cannot completely exclude the possibility of other causative drugs. Another possibility is that *HLA-B*1502* is more prone than *HLA-B*1511* to cause carbamazepine-induced SJS/TEN. Carbamazepine or its metabolites may covalently (Weltzien et al., 1996) or noncovalently (Wu et al., 2007; Yang et al., 2007) bind more easily to the HLA-B*1502 protein or its binding peptide.

There are no SJS/TEN patients carrying *HLA-B*1511* who had severe ocular complications. This result coincides with the previous report that none of the 71 SJS/TEN patients with ocular surface complications had *HLA-B*1511* (Ueta et al., 2008).

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Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflict of interest to disclose.

References

Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. (1993) Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch Dermatol* 129:92–96. The diagnostic criteria are reflected in the currently used following Japanese guidance; Diagnostic criteria of Stevens–Johnson syndrome (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005) and Diagnostic criteria of Toxic Epidermal Necrolysis (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005).

Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, Chen YT. (2004) Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 428:486.

Ikeda H, Takahashi Y, Yamazaki E, Fujiwara T, Kaniwa N, Saito Y, Aihara M, Kashiwagi M, Muramatsu M. (2010) HLA Class I markers in

Japanese patients with carbamazepine-induced cutaneous adverse reactions. *Epilepsia* 51:297–300.

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

Locharernkul C, Loplumlert J, Limotai C, Korkij W, Desudchit T, Tongkobpetch S, Kangwanshiratada O, Hirankarn N, Suphapeetiporn K, Shotelersuk V. (2008) Carbamazepine and phenytoin induced Stevens–Johnson syndrome is associated with HLA-B*1502 allele in Thai population. *Epilepsia* 49:2087–2091.

Lonjou C, Thomas L, Borot N, Ledger N, de Toma C, LeLouet H, Graf E, Schumacher M, Hovnanian A, Mockenhaupt M, Roujeau JC; RegiSCAR Group. (2006) A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 18:99–107.

Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, Ng MH. (2007) Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48:1015–1018.

Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, Dave DM, Goyal RK. (2009) Association of HLA-B*1502 allele and carbamazepine-induced Stevens–Johnson syndrome among Indians. *Indian J Dermatol Venereol Leprol* 75:579–582.

Middleton D, Menchaca L, Rood H, Komerofsky R. (2003) Brief communication. New allele frequency database: http://www.allelefrequencies.net. *Tissue Antigens* 61:403–407.

Tanaka H, Akaza T, Juji T. (1996) Report of the Japanese central bone marrow data center. *Clin Transpl* 1996:139–144.

Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Chen P, Lin SY, Chen WH, Konyoung P, Khunarkornsiri U, Auvichayapat N, Pavakul K, Kulkantrakorn K, Choonhakarn C, Phonhiamhan S, Piyatrakul N, Aungaree T, Pongpakdee S, Yodnopaglaw P. (2010) Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. *Epilepsia* 51:926–930.

Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S. (2008) HLA class I and II gene polymorphisms in Stevens–Johnson syndrome with ocular complications in Japanese. *Mol Vis* 14:550–555.

Weltzien HU, Moulton C, Martin S, Padovan E, Hartmann U, Kohler J. (1996) T cell immune responses to haptens. Structural models for allergic and autoimmune reactions. *Toxicology* 107:141–151.

Wu Y, Farrell J, Pirmohamed M, Park BK, Naisbitt DJ. (2007) Generation and characterization of antigen-specific CD4 + , CD8 + , and CD4 + CD8 + T-cell clones from patients with carbamazepine hypersensitivity. *J Allergy Clin Immunol* 119:973–981.

Yang CW, Hung SI, Juo CG, Lin YP, Fang WH, Lu IH, Chen ST, Chen YT. (2007) HLA-B*1502-bound peptides: implications for the pathogenesis of carbamazepine-induced Stevens–Johnson syndrome. *J Allergy Clin Immunol* 120:870–877.

Yang G, Deng YJ, Qin H, Zhu BF, Chen F, Shen CM, Sun ZM, Chen LP, Wu J, Mu HF, Lucas R. (2010) HLA-B*15 subtypes distribution in Han population in Beijing, China, as compared with those of other populations. *Int J Immunogenet* 37:205–212.

Long-Term Phenotypic Study after Allogeneic Cultivated Corneal Limbal Epithelial Transplantation for Severe Ocular Surface Diseases

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Purpose: To determine the long-term epithelial lineage of origin of surgically removed grafts after allogeneic cultivated corneal limbal epithelial transplantation (CLET).

Design: Interventional case reports.

Participants: We studied 2 eyes from 2 patients with total corneal stem cell destruction; 1 eye was from a patient with Stevens-Johnson syndrome and 1 eye had sustained chemical injury.

Methods: Allogeneic cultivated corneal limbal epithelial sheets on human amniotic membrane (AM) were transplanted onto the ocular surface. Regrafting (1 eye, 42 months later) or penetrating keratoplasty (1 eye, 75 months later) were performed after the initial transplantation procedure for further visual rehabilitation.

Main Outcome Measures: The excised grafts were subjected to clinical evaluation and to light- and transmission electron microscopy (TEM) examination and to immunohistochemical analysis.

Results: In clinically conjunctival grafts, TEM and immunohistochemical analysis disclosed only small areas where the original cultivated corneal epithelial cells persisted. Neighboring conjunctival epithelial cells had apparently invaded a large portion of the corneal surface (keratin 3/12(−), Muc5ac(+)). In clinically corneal grafts, transplanted allogeneic cultivated corneal epithelial cells clearly survived for a long period of time (keratin 3/12(+), Muc5ac(−)); there was no infiltration by inflammatory cells, nor was there dissolution of the AM substrate.

Conclusions: We theorize that the process of graft opacification after allogeneic CLET is responsible for the loss of transplanted cultivated corneal epithelial cells and that this is followed by conjunctival cell invasion onto the corneal surface. The results of this study confirmed that in the clinically evaluated corneal graft, transplanted cultivated corneal epithelial cells indeed survived for a long period of time on the corneal surface and maintained ocular surface integrity, even though the transplanted cells were allogeneic.

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Severe ocular surface diseases (OSD), such as Stevens-Johnson syndrome and chemical burns, are currently some of the most challenging of disorders. These conditions are characterized by the destruction of corneal epithelial stem cells in the limbus that results in conjunctivalization, neovascularization, chronic inflammation, and corneal stromal scarring.[1–3] Conventional corneal transplantation has proven to provide less-than-satisfactory clinical results in patients with severe OSD.[4,5] Recently, attention has focused on the development of regenerative cell therapy such as tissue-engineered cultivated epithelial stem cell transplantation as a new approach for ocular surface reconstruction in cases of severe OSD.[6–9] For treating severe OSD, it is necessary to transplant an autologous or allogeneic cell source to restore the normal ocular surface. In fact, most severe OSDs are bilateral, thus forcing surgeons to use allograft donor cells, which subject the recipients to a high risk for allogeneic rejection.

Since 1999, we have performed allogeneic cultivated corneal limbal epithelial transplantation (CLET) using human amniotic membrane (AM) as a carrier on 51 human eyes with the acute- or chronic-phase of severe OSD.[8,10] Although our initial clinical assessments of allogeneic CLET yielded favorable results from the perspective of ocular surface stabilization, longevity and long-term phenotypic analyses of allogeneic corneal epithelial transplants to the ocular surface must be performed because there have been no reports regarding these subjects. Because it has not been determined what happens to allogeneic cultivated transplants on the human ocular surface, we compared our

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clinical observations with the results of long-term cellular phenotype analysis of allogeneic CLET.

Herein we have reported for the first time our long-term clinical, histologic, ultrastructural, and immunohistochemical findings on allogeneic CLET in detail. Our findings have important clinical implications and provide valuable insights into the mechanisms of both graft survival and graft integrity after allogeneic CLET.

Materials and Methods

Subjects

All experimental procedures and clinical applications introduced here were approved by the Institutional Review Board for Human Studies of Kyoto Prefectural University of Medicine; prior informed consent was obtained from all patients in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. Our study included 2 eyes from 2 patients; 1 patient with Stevens-Johnson syndrome (patient 1, right eye) and 1 patient with chemical injury (patient 2, left eye). Patient 1 is a 35-year-old woman and patient 2, a 63-year-old man. Preoperatively, all eyes manifested severe destruction of the ocular surface with total limbal stem cell deficiency. These patients demonstrated a reasonable reflex tear function and tear meniscus level, and both patients had initially undergone allogeneic CLET to reconstruct the ocular surface. Postoperatively, 0.3% ofloxacin and 0.1% dexamethasone eye drops were instilled 4 times a day. The doses were tapered to a maintenance dose of 2 to 3 times per day after 2 to 3 months, depending on the severity of inflammation. Systemic betamethasone (1 mg/d), cyclophosphamide (50 mg/d), and cyclosporine (100 mg/d) were administered orally to reduce postoperative inflammation, scarring, and allograft rejection. Renal and liver functions were monitored periodically. Administration of the former 2 immunosuppressive agents were stopped between 1 and 3 months after surgery.

Patient 1. The onset of the disease was when the patient was 2 or 3 years old. We could not follow the patient's clinical course after disease onset in detail, but 33 years later, she visited our clinic and both of her eyes showed total corneal epithelial stem cell deficiency with total conjunctivalization, inflammation, symblepharon, and neovascularization. For this patient, we first reconstructed the ocular surface by allogeneic CLET combined with AM transplantation. During the postoperative month 21, the patient's corneal surface was completely reconstructed by a transplanted allogeneic CLET graft; however, preexisting intrastromal neovascularization was still observed in the corneal stroma. Best-corrected visual acuity was improved from 20/100 to 20/32. Subsequently, there was a recurrence of minimal cell infiltration and inflammation in the upper part of the patient's corneal and conjunctival areas. Even though the patient's ocular surface was stable without any epithelial defects, the same clinical appearances sometimes recurred and conjunctiva gradually invaded into the central part of the cornea. We therefore transplanted the allogeneic cultivated corneal limbal epithelial sheet again 42 months after the initial allogeneic CLET to further visual rehabilitation.

Patient 2. The patient suffered chemical injury to both eyes at age 63. Although both eyes positively responded to the medical treatment, they ultimately showed total corneal epithelial stem cell deficiency with total conjunctivalization, inflammation, symblepharon, and neovascularization. For this patient, we first reconstructed the ocular surface by allogeneic CLET combined with AM transplantation. The elapsed time from disease onset to the first graft was 10 months. During postoperative month 19, the patient's corneal surface was completely reconstructed by a transplanted allogeneic CLET graft; however, the preexisting diffuse intrastromal opacity was still observed. Best-corrected visual acuity was improved from counting fingers to 8/200. Subsequently, the patient's ocular surface was apparently stable without any epithelial defects; however, peripheral conjunctivalization gradually occurred. Severe preoperative corneal stromal opacity that strongly affected the patient's visual acuity prompted us to perform penetrating keratoplasty 75 months after the initial allogeneic CLET.

Opaque cultured epithelium (patient 1) and corneal buttons (patient 2) harvested from these eyes at the time of the second transplantation were processed for light and electron microscopy and for immunohistochemical study. The operative procedures introduced herein followed previously reported methods.[8,10]

Examination by Transmission Electron Microscopy

Samples from the allogeneic CLET grafts were examined by transmission electron microscopy (TEM). The specimens were fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate-buffered saline (PBS), washed 3 times for 15 minutes each in PBS, post-fixed for 2 hours in 2% aqueous osmium tetroxide, washed 3 more times in PBS, and then passed through a graded ethanol series (50%, 70%, 80%, 90%, 95%, and 100%). For TEM (JEM 1010; JEOL Ltd., Tokyo, Japan) examination, the specimens were embedded in araldite resin (Agar Scientific Ltd., Stansted, UK), ultrathin (70-nm) sections were placed on copper grids, stained for 1 hour with uranyl acetate and 1% phosphotungstic acid, and then for 20 minutes with Reynold's lead citrate.

Immunohistochemistry

Immunohistochemical studies to detect tissue-specific keratins in the removed grafts were performed in accordance with our previously described method.[11-13] Briefly, 7-μm-thick cryostat sections were placed on gelatin-coated slides, air dried, and rehydrated in PBS for 15 minutes at room temperature (RT). To block nonspecific binding, the tissues were incubated for 30 minutes with 2% bovine serum albumin at RT. They were then incubated at RT for 1 hour with the appropriate primary antibodies (Table 1; available online at http://aaojournal.org). They were subsequently washed 2 times in PBS containing 0.15% TritonX-100 for 15 minutes. Control incubations were performed with the appropriate normal mouse- and rabbit immunoglobulin (Ig)G (Dako, Kyoto, Japan) at the same concentration as the primary antibody, but without antibody. After staining with the primary antibody, the sections were incubated at RT for 1 hour with the appropriate secondary antibodies and FITC-conjugated donkey anti-mouse IgG and FITC-conjugated goat anti-rabbit IgG (Molecular Probes Inc., Eugene, OR), respectively. After several washings with PBS, the sections were coverslipped using antifading mounting medium containing propidium iodide (Vectashield; Vector Laboratories, Burlingame, CA) and examined under a confocal microscope (Olympus Fluoview, Tokyo, Japan).

Results

Clinical and Histologic Findings

Patient 1. Clinically, slit-lamp examination showed that the upper and temporal part of the corneal area was apparently covered with conjunctival epithelium (conjunctival phenotype; yellow circle E and G in Fig 1A); however, the lower and nasal part of the corneal

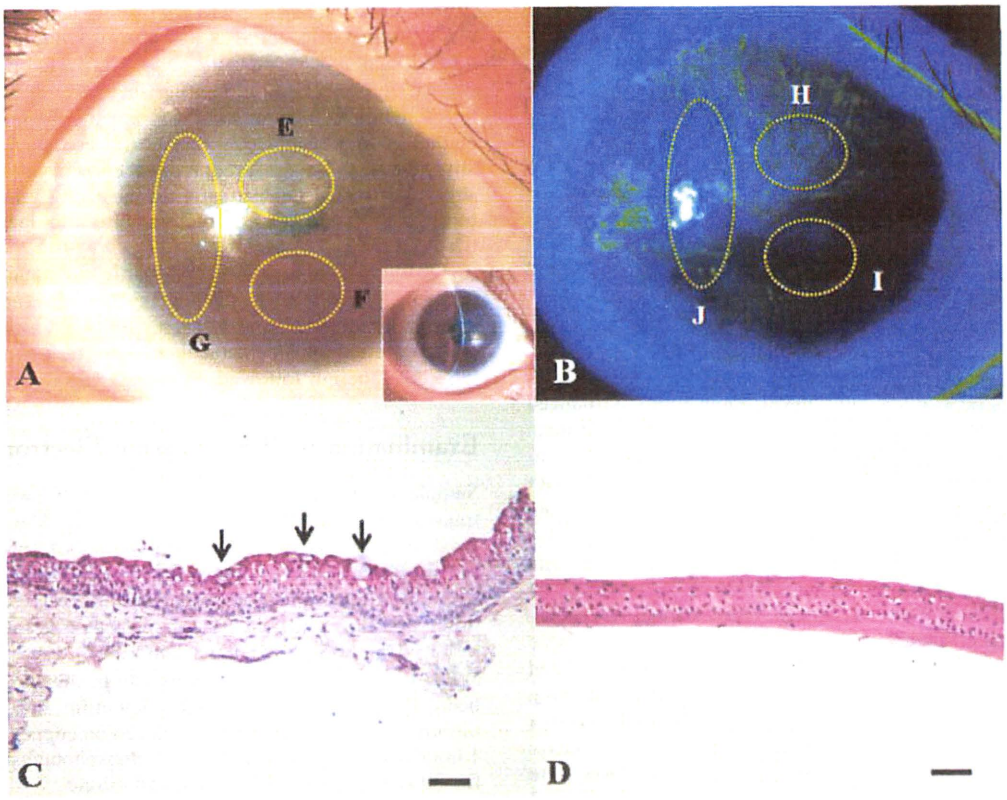

Figure 1. Representative slit-lamp photographs before (A) and after (A; inset) allogeneic cultivated corneal limbal epithelial transplantation in patient 1. Slit-lamp examination revealed that the upper and temporal part of the corneal surface was apparently covered with conjunctiva (A; yellow circles E and G). In contrast, slit-lamp examination revealed that the lower nasal part of the corneal surface was still transparent (A; yellow circle F). Fluorescein staining showed a comparatively clear demarcation between corneal and conjunctival phenotypes (B). The corneal phenotype area was comparatively smooth epithelium with no fluorescein staining (B; yellow circle I), yet the conjunctival phenotype area revealed light and stippled staining with fluorescein (B; yellow circles H and J). The cross-section of yellow circle E disclosed 5 to 6 stratified layers of conjunctivallike epithelial cells and also included the goblet cell–like cells (black arrows; C). The cross-section of yellow circle F showed 5 to 6 stratified cell layers and cornealike epithelial cells (D). Amniotic membrane substrate was clearly observed throughout the epithelium and there were no inflammatory cells. Scale bars (C, D) = 50 μm.

area remained transparent and apparently covered by the transplanted allogeneic cultivated corneal limbal epithelial sheet (corneal phenotype; yellow circle F in Fig 1A). Fluorescein staining showed a comparatively clear demarcation between the corneal and conjunctival phenotypes (Fig 1B). The corneal phenotype area was comparatively smooth epithelium and there was no fluorescein staining (yellow circle I in Fig 1B), but the conjunctival phenotype area revealed light and stippled staining with fluorescein (yellow circle H and J in Fig 1B). Light microscopic examination of the removed cultured sheet of the conjunctival phenotype (yellow circle E in Fig 1A) disclosed 5 to 6 stratified cell layers and conjunctival-like epithelial cells (Fig 1C). The removed cultured sheet also included the goblet-cell–like cells (black arrows in Fig 1C). The AM substrate was not clearly observed throughout the epithelium. In contrast, light microscopic examination of the removed cultured sheet of the corneal phenotype (yellow circle F in Fig 1A) showed 5 to 6 stratified cell layers, and basal cells were flattened to the superficial layers (Fig 1D). The AM substrate was clearly observed throughout the epithelium and there were no fibroblastic tissues or accompanying inflammatory cells.

Patient 2. Clinically, slit-lamp examination clearly revealed diffuse intrastromal opacity and peripheral conjunctivalization (Fig 2A). Fluorescein staining showed that the central part of the cornea was comparatively smooth epithelium with no staining (corneal phenotype; yellow circle E in Fig 2B), but the peripheral region revealed light and stippled staining with fluorescein (conjunctival phenotype; yellow circle F in Fig 2B). Light microscopic examination of the removed corneal buttons in the conjunctival phenotype disclosed 5 to 6 stratified cell layers and conjunctival-like epithelial cells (Fig 2C). The AM substrate was not clearly observed throughout the epithelium. In contrast, light microscopic examination of the corneal phenotype showed 5 to 6, well-formed, stratified cell layers (Fig 2D). The AM substrate was clearly observed throughout the epithelium and there were few inflammatory cells.

Immunohistochemistry

We investigated the expression patterns of tissue-specific keratins in samples from both grafts. Negative control sections, incubated with normal mouse and rabbit IgG in the absence of primary antibody, exhibited no discernible specific immunoreactivity. We compared control samples and graft specimens prepared for immunohistochemistry.

In specimens from the conjunctival phenotypic area of patient 1, epidermal-keratinization–specific keratins 1 and 10 were not observed in any epithelial layers (Fig 3A, B). The nonkeratinized, stratification-specific keratins 4 and 13 were expressed in all epithelial layers except the basal cell layer (Fig 3E, F); cornea-specific keratins 3 and 12 were not observed in any of the epithelial

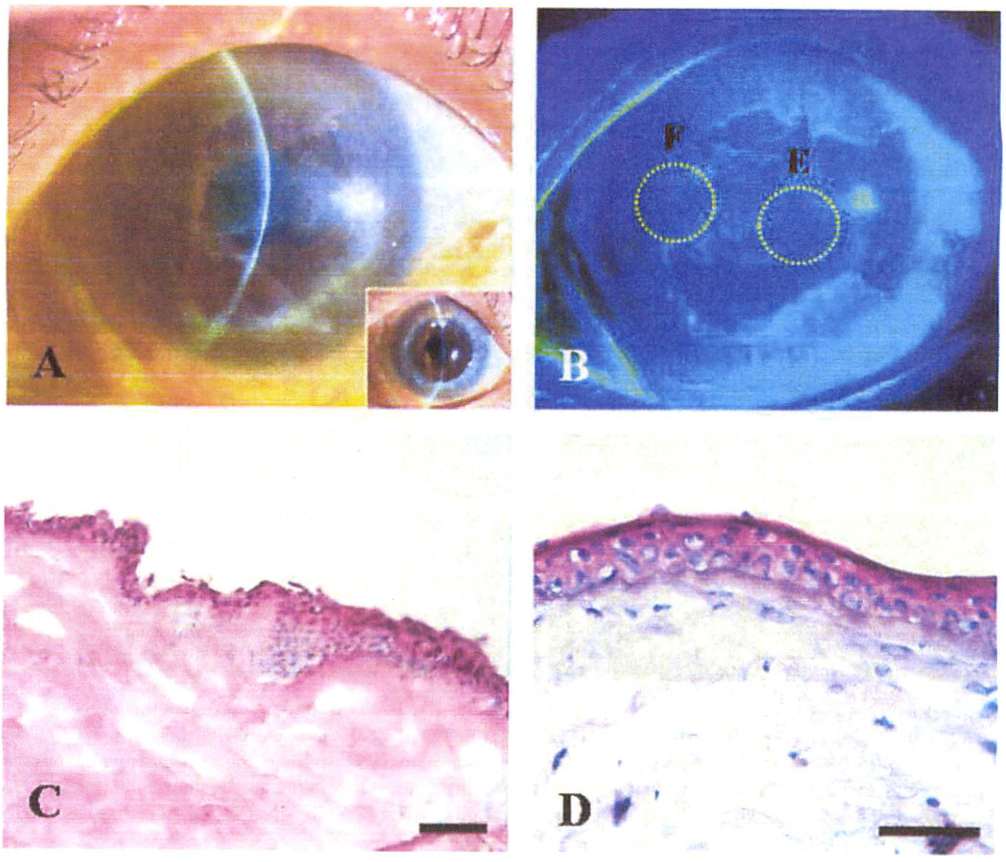

Figure 2. Representative slit-lamp photographs before (A) and after (A; inset) allogeneic cultivated corneal limbal epithelial transplantation in patient 2. Slit-lamp examination clearly revealed diffuse intrastromal opacity and peripheral conjunctivalization (B). Fluorescein staining showed that the central part of the cornea was comparatively smooth epithelium (B; yellow circle E), but the peripheral region revealed the conjunctival staining pattern (B; yellow circle F). The cross section of yellow circle C disclosed 5 to 6 stratified cell layers and conjunctival-like epithelial cells (C). Amniotic membrane (AM) substrate was not clearly observed throughout the epithelium. In contrast, the cross section of yellow circle D showed 5 to 6 well-formed stratified cell layers and cornealike epithelial cells (D). AM substrate was clearly observed throughout the epithelium. Scale bars (C, D) = 50 μm.

layers (Fig 3I, J). Limbal–conjunctival basal cell marker keratin 15 and goblet cell marker Muc5ac were sporadically expressed in the removed culture sheet (Fig 3M, N). In contrast, in specimens from the corneal phenotypic area of patient 1, keratins 1/10 and 4/13 were not observed in any of the epithelial layers (Fig 3C, D, G, H). The cornea-specific keratins 3 and 12 were present in almost all epithelial layers (Fig 3K, L). Keratin 15 was sporadically expressed in some areas (Fig 3O), but there was no localization with Muc5ac (Fig 3P). In addition, we further examined the temporal part of the specimens, which is clinically thought to be the conjunctival phenotype (yellow circle G in Fig 1A), and found that cornea-specific keratins 3 and 12 were unexpectedly expressed throughout the epithelium (Fig 4; available online at http://aaojournal.org).

In specimens from the conjunctival phenotypic area of patient 2, keratins 1 and 10 were also not observed in any of the epithelial layers (Fig 5A, B). Keratins 4 and 13 were clearly expressed in the epithelial layer (Fig 5E, F), and faint keratin 3 and 12 staining was observed in the epithelial layer (Fig 5I, J). Keratin 15 and Muc5ac were sporadically expressed in the epithelial layer (Fig 5M, N). In contrast, in specimens from the corneal phenotypic area of patient 2, keratins 1/10 and 4/13 were barely observable in any of the epithelial layers (Fig 5C, D, G, H). The cornea-specific keratins 3 and 12 were clearly present in almost all epithelial layers (Fig 5K, L). Keratin 15 was sporadically expressed in some areas (Fig 5O), but there was no localization with Muc5ac (Fig 5P). The results of the immunohistochemical analyses are summarized in Table 2.

Transmission Electron Microscopy

Using TEM, we carefully examined the ultrastructural differences between the clinically evaluated conjunctival and corneal areas in patient 1. TEM examination showed that the clinically evaluated conjunctival area contained cells of a conjunctival origin, including numerous goblet cells which were easy to identify (Fig 6A, B). The goblet cells contained numerous mucin granules with dense cytoplasm within them and long microvilli on their surfaces. In some regions the conjunctival cells had migrated over cells with a more corneallike phenotype. Cells with the corneallike phenotype were 10 to 12 microns in size, columnar or cuboid in shape, with tightly opposed cell borders and round nuclei. In contrast, cells with the conjunctival phenotype were 6 to 8 microns in size and irregular in shape, with densely staining cytoplasm, intercellular spaces, and oval or flattened nuclei. In other regions, the conjunctival cells were growing directly on the AM. TEM examination showed that the clinical corneal area contained cells with a corneal epithelial phenotype, and that the basal wing and superficial cells in this region seemed normal with no signs of necrosis or apoptosis (Fig 6C, D). In all cell layers, the epithelial cells were comparatively closely attached to neighboring cells by numerous desmosomal junctions. The basal epithelial cells adhered well to the AM substrate via hemidesmosome attachments and a basement membrane was present (data not shown). The stromal region contained numerous keratocytes.

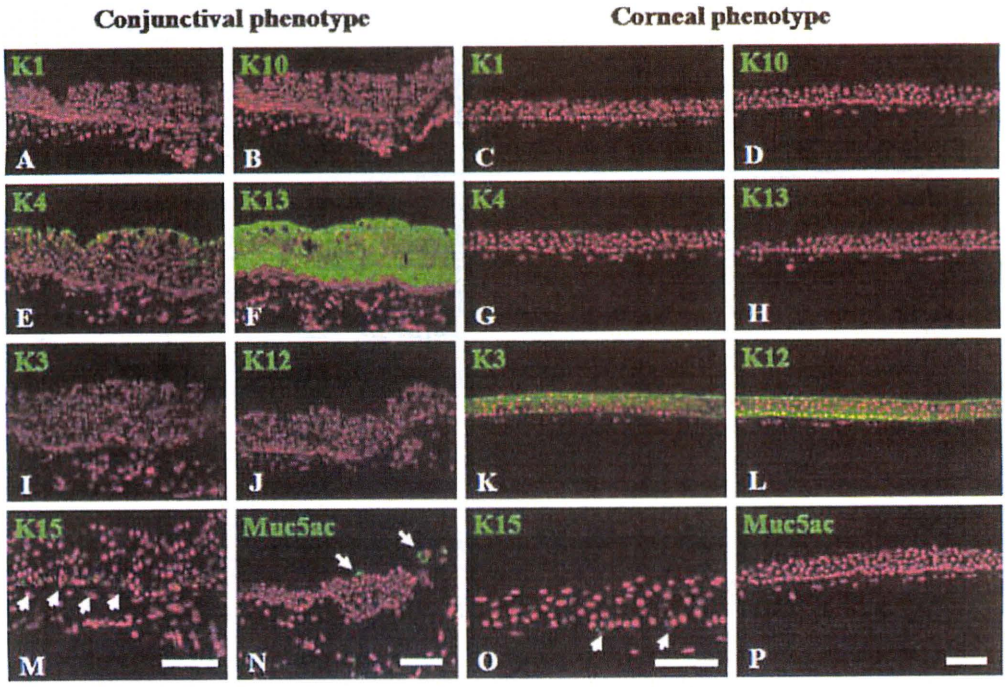

Figure 3. In the conjunctival phenotypic area of patient 1, keratins 1 and 10 were not observed in any of the epithelial layers (A, B). Keratins 4 and 13 were expressed in all epithelial layers except the basal cell layer (E, F); keratins 3 and 12 were not observed in any of the epithelial layers (I, J). Keratin 15 and Muc5ac were sporadically expressed in the removed culture sheet (white arrows; M, N). In the corneal phenotypic area of patient 1, keratins 1/10 and 4/13 were not observed in any of the epithelial layers (C, D, G, H). Keratins 3 and 12 were present in almost all epithelial layers (K, L). Keratin 15 was sporadically expressed in some areas (white arrows; O), but there was no localization with Muc5ac (P). Scale bars = 100 μm.

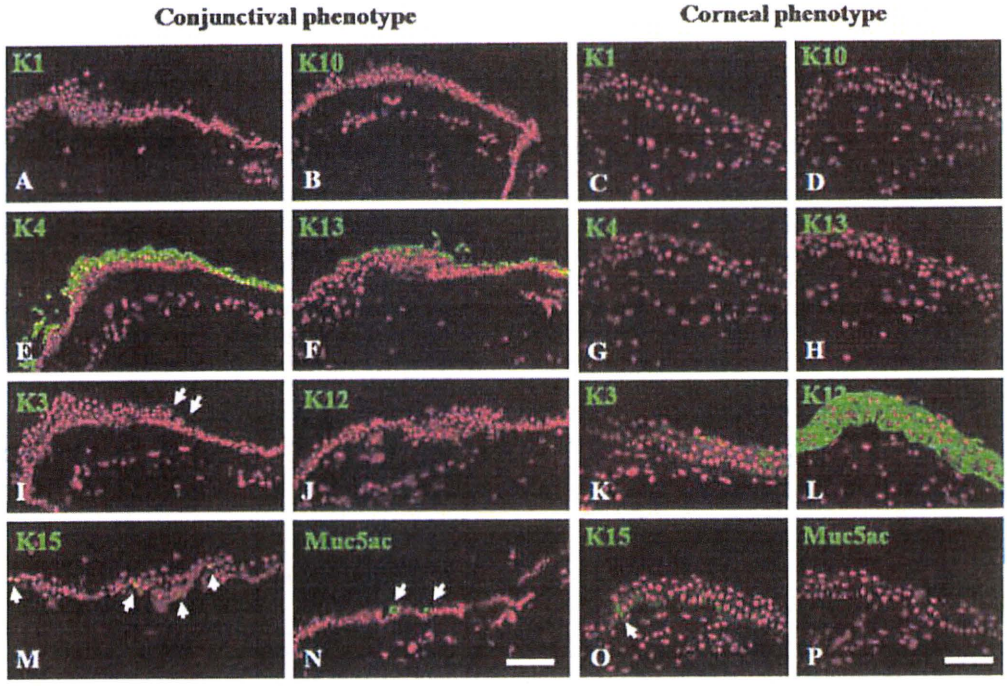

Figure 5. In the conjunctival phenotypic area of patient 2, keratins 1 and 10 were not observed in any of the epithelial layers (A, B). Keratins 4 and 13 were clearly expressed in the epithelial layer (E, F). Faint keratin 3 and 12 staining was observed in the epithelial layer (I, J). Keratin 15 and Muc5ac were sporadically expressed in the epithelial layer (M, N). In specimens from the corneal phenotypic area of patient 2, keratins 1/10 and 4/13 were barely observable in any of the epithelial layers (C, D, G, H). The cornea-specific keratins 3 and 12 were clearly present in almost all of the epithelial layers (K, L). Keratin 15 was sporadically expressed in some areas (O), but there was no localization with Muc5ac (P). Scale bars = 100 μm.

Table 2. Summary of Immunohistochemical Results

	K1	K10	K4	K13	K3	K12	K15	Muc5ac
Case 1								
Corneal phenotype	−	−	±	−	+	+	+	−
Conjunctival phenotype	−	−	+	+	−	−	+	+
Case 2								
Corneal phenotype	−	−	±	−	+	+	+	−
Conjunctival phenotype	−	−	+	+	±	±	+	+

Discussion

We have previously demonstrated that allogeneic CLET holds promise as a novel operative treatment for severe OSD.[8,10] This new operative modality requires long-term postoperative immune suppression; therefore, there is a high risk for postoperative cultured graft rejection. There have been various reports regarding the phenotypic analysis after CLET, yet those studies only reported a short-term phenotypic analysis (3–12 months).[14–16] At present, the long-term morphologic and biological corneal phenotypes after allogeneic CLET are not fully understood. Herein we have demonstrated for the first time that our clinical slit-lamp findings were almost consistent with the morphologic and cell biological phenotypes. Moreover, using the proper postoperative treatment, tissue engineered cultivated corneal cells can partially survive for a long period of time on the ocular surface, even though the cell origin is allogeneic.

In the cases in this present study, the ocular surface was initially reconstructed with allogeneic cultivated corneal epithelium, but owing to postoperative ocular surface conditions, the corneal surface was eventually opaque and required reconstruction of the ocular surface to improve the visual acuity. At that time, we did not know precisely

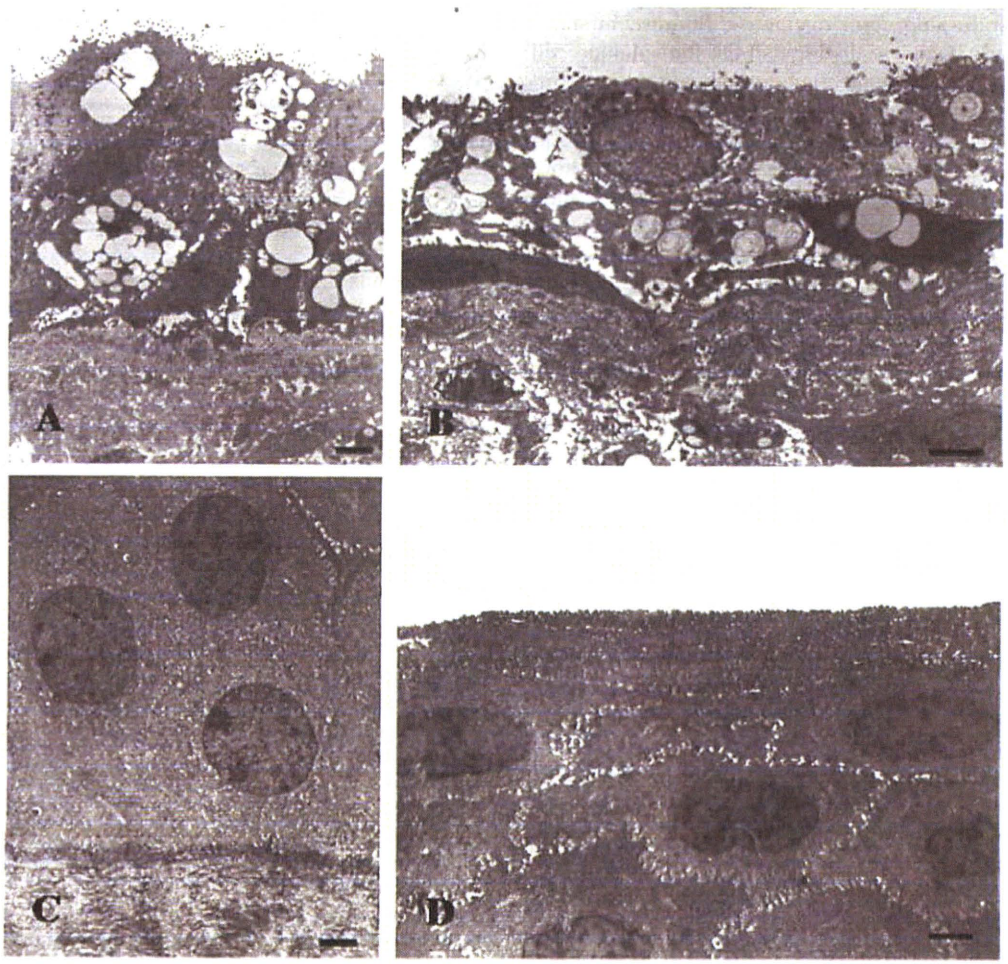

Figure 6. Transmission electron microscopy (TEM) examination (Patient 1) showed that the phenotypic conjunctival area contained cells of conjunctival origin including numerous goblet cells, which were easy to identify (A, B). The goblet cells contained numerous mucin granules with dense cytoplasm within them and long microvilli on their surfaces. Examination by TEM showed that the phenotypic corneal area contained cells with a corneal epithelial phenotype, and that the basal wing and superficial cells in this region seemed normal with no signs of necrosis or apoptosis (C, D). In all cell layers the epithelial cells were closely attached to neighboring cells by numerous desmosomal junctions. Scale bars = 2 μm.

whether the opaque epithelium (clinically evaluated conjunctival phenotype) was replaced by surrounding conjunctival epithelial cells or whether the initially transplanted cultured corneal sheet itself became opaque. Analysis by TEM showed only a small area of the graft where the original cultivated corneal epithelial cells remained; however, it seemed that neighboring conjunctival epithelial cells had mainly invaded the corneal surface. Immunohistochemical analysis showed that the expression patterns of tissue-specific keratins in the clinically evaluated conjunctival phenotypic grafts were similar to those in the conjunctival epithelium (mucosa-specific keratin 4/13(+), cornea-specific keratin 3/12(−), and Muc5ac (+)). Our clinical, ultrastructural, and cell biological findings revealed that the process of graft opacification was responsible for the loss of transplanted cultivated corneal epithelial cells and that this event was followed by surrounding conjunctival invasion onto the corneal surface.

In contrast, even though the transplanted cultured sheet showed the conjunctival phenotype in some areas, the clinically transparent areas in the corneal surface (corneal phenotype) were still observable. During the course of the postoperative follow-up, their distinctive fluorescein staining patterns made it easy to distinguish the transplanted cell sheets from the surrounding conjunctival epithelium. Immunohistochemical analysis showed that the expression patterns of the tissue-specific keratins in the clinically evaluated corneal phenotypic grafts were consistent with those seen in normal corneal epithelium (keratin 3(+), keratin 12(+), and Muc5ac(−)). Unfortunately, we were unable to examine the original allogeneic cultivated corneal limbal epithelium before transplantation due to the ethical considerations and clinical matters, however, and because we indicated in a previous report, allogeneic cultivated corneal epithelial sheets normally show keratin 3/12(+) and Muc5ac(−), which are identical staining patterns to that of corneal phenotypic areas.[10,12] This set of findings confirmed that transplanted allogeneic cultivated corneal epithelial cells can survive on the corneal surface and maintain ocular surface integrity for a long period of time, indicating that this allogeneic system may prove to be a potentially useful operative tool for ocular surface reconstruction. To that regard, proper postoperative immunosuppressive therapy is very important for the survival of this graft.[17] We noted that in our series, the 1 patient who had received the allo-CLET operation 10 years ago still maintained corneal transparency, thus reinforcing our decision to perform this type of transplantation.

For successful cultivated corneal epithelial transplantation, it is essential to involve corneal stem/progenitor cells in the cultivated sheet if long-term graft survival is expected. It has been reported that keratin 15 is a marker for corneal limbal and conjunctival basal cells, indicating that it may also become the marker for corneal limbal stem/progenitor cells.[18] In general, proliferation occurs in the basal layer of keratinocyte stem cells attached to the underlying basement membrane. Stem cells facilitate the maintenance of self-renewing tissues; they are critical for replenishing and maintaining the cell balance within tissues. Interestingly, our immunohistochemical studies detected the sporadic expression of keratin 15 in very small restricted areas in all eyes. In view of these findings, although it is still too early to demonstrate that stem cells of an allogeneic cultivated corneal epithelial sheet persist in the ocular surface for a long time after transplantation, it does imply the long-term survival of transplanted allogeneic stem/progenitor cells. Studies are currently underway in our laboratory to shed further light of this observation.

In conclusion, we examined the long-term morphologic and cell biological phenotypes of allogeneic CLET grafts. We found that in clinical conjunctival phenotypic grafts, transplanted cultivated corneal epithelial cells were gradually replaced by surrounding conjunctival epithelial cells and we noted the presence of many inflammatory cells. Clinical corneal phenotypic grafts demonstrated that transplanted cultivated corneal cells were able to survive for a long period of time and become integrated into the host corneal stroma. The current immunohistochemical and ultrastructural examination yields novel information on allogeneic CLET and suggests reasons for the phenotypic diversities of these transplants. We are currently using this information in our attempt to develop more effective postoperative therapies for patients with severe OSD.

References

1. Tseng SC. Concept and application of limbal stem cells. Eye (Lond) 1989;3:141–57.
2. Dua H, Forrester JV. The corneoscleral limbus in human corneal epithelial wound healing. Am J Ophthalmol 1990;110: 646–56.
3. Kinoshita S, Adachi W, Sotozono C, et al. Characteristics of the human ocular surface epithelium. Prog Retin Eye Res 2001;20:639–73.
4. Thoft RA. Keratoepithelioplasty. Am J Ophthalmol 1984;97:1–6.
5. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–22; discussion 722–3.
6. Pellegrini G, Traverso CE, Franzi AT, et al. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 1997;349:990–3.
7. Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 2000;343:86–93.
8. Koizumi N, Inatomi T, Suzuki T, et al. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 2001;108:1569–74.
9. Shimazaki J, Aiba M, Goto E, et al. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. Ophthalmology 2002;109:1285–90.
10. Nakamura T, Inatomi T, Sotozono C, et al. Transplantation of autologous serum-derived cultivated corneal epithelial equivalents for the treatment of severe ocular surface disease. Ophthalmology 2006;113:1765–72.
11. Nakamura T, Endo K, Cooper LJ, et al. The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. Invest Ophthalmol Vis Sci 2003;44:106–16.
12. Nakamura T, Ang LP, Rigby H, et al. The use of autologous serum in the development of corneal and oral epithelial equivalents in patients with Stevens-Johnson syndrome. Invest Ophthalmol Vis Sci 2006;47:909–16.

13. Nakamura T, Inatomi T, Cooper LJ, et al. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe surface diseases. Ophthalmology 2007;114:1080–8.
14. Grueterich M, Espana EM, Touhami A, et al. Phenotypic study of a case with successful transplantation of ex vivo expanded human limbal epithelium for unilateral total limbal stem cell deficiency. Ophthalmology 2002;109:1547–52.
15. Cooper LJ, Fullwood NJ, Koizumi N, et al. An investigation of removed cultivated epithelial transplants in patients after alloculti-vated corneal epithelial transplantation. Cornea 2004;23:235–42.
16. Kawashima M, Kawakita T, Satake Y, et al. Phenotypic study after cultivated limbal epithelial transplantation for limbal stem cell deficiency. Arch Ophthalmol 2007;125:1337–44.
17. Maruyama K, Yamada J, Sano Y, Kinoshita S. Th2-biased immune system promotion of allogeneic corneal epithelial cell survival after orthotopic limbal transplantation. Invest Ophthalmol Vis Sci 2003;44:4736–41.
18. Yoshida S, Shimmura S, Kawakita T, et al. Cytokeratin 15 can be used to identify the limbal phenotype in normal and diseased ocular surfaces. Invest Ophthalmol Vis Sci 2006;47:4780–6.

Footnotes and Financial Disclosures

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Table 1. Primary Antibodies and Source

Antibodies	Category	Dilution	Source
Keratin 1	Mouse monoclonal	X100	Novocastra, UK
Keratin 3	Mouse monoclonal	X100	Progen, Germany
Keratin 4	Mouse monoclonal	X200	Novocastra, UK
Keratin 10	Mouse monoclonal	X200	Novocastra, UK
Keratin 12	Rabbit polyclonal	X200	Trangenic, Japan
Keratin 13	Mouse monoclonal	X200	Novocastra, UK
Keratin 15	Mouse monoclonal	X200	Abcam, UK
Muc5ac	Mouse monoclonal	X100	Zymed, USA

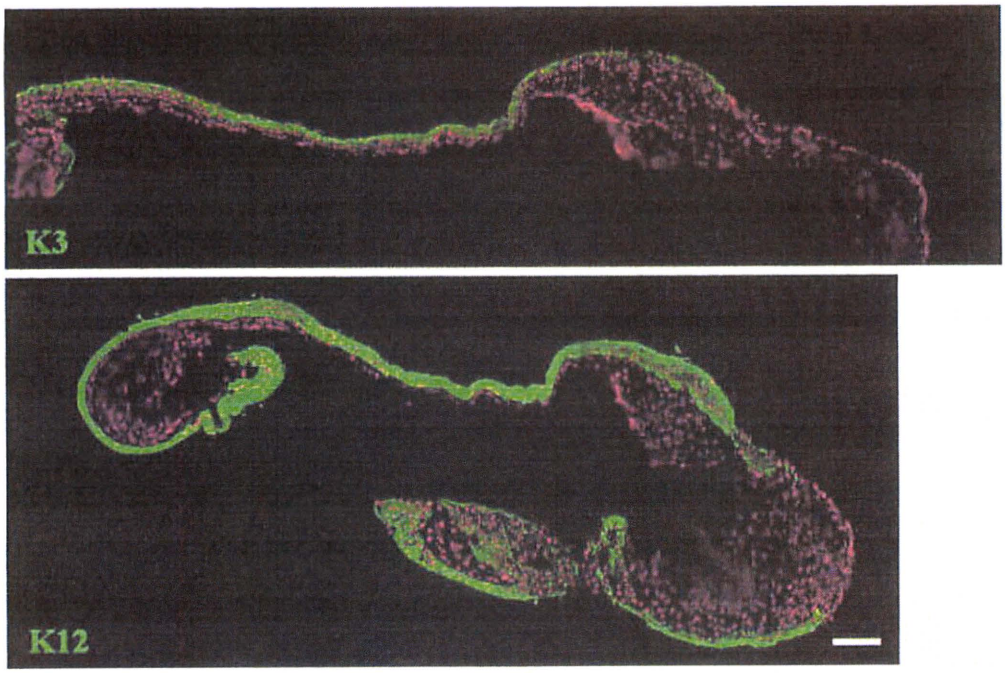


Figure 4. In the temporal part of the specimens, which is clinically thought to be the conjunctival phenotype, cornea-specific keratins 3 and 12 were unexpectedly expressed throughout the epithelium. Scale bars = 100 μm.

重症薬疹では眼病変に注意

上田真由美/外園千恵

What's new?

重症薬疹とは，全身の皮膚病変に加えて粘膜疹，臓器障害を併発しやすく，生命に危険を及ぼす可能性のある薬疹であり，スティーブンス・ジョンソン症候群(SJS)，中毒性表皮壊死症(TEN)，薬剤過敏症症候群(DIHS)などが含まれる．このなかで，眼科と最も関連が深いのは，SJSならびにその重症型とされるTENである．SJS/TENにおける眼合併症率は，およそ60％と報告されており[1]，眼科的にはSJSとTENによる所見の違いはない．薬疹発症後に眼表面が瘢痕化すると，重篤な視力障害のほか，著しいドライアイ，睫毛乱生が生涯にわたって持続する(図1)[2]．このような重篤な眼合併症を伴うSJS/TEN患者では，発症時に急性結膜炎，口唇・口腔内の出血性びらん，爪囲炎を必発することがわかってきた(図2)[3]．また，眼合併症を伴う患者では感冒様症状が薬剤投与に先行することが多い[4]．発症時から眼合併症の有無を判断し，ステロイドによる強力な消炎治療を行うことが，眼科的予後を改善する．

Essence

眼合併症を伴うSJS/TENの急性期には，皮疹・粘膜疹とほぼ同時に，または数日程度先行して，両眼性に急性結膜炎を生じる．言い換えると，皮膚に発疹を生じた時点で両眼が充血している場合は，眼合併症を伴う可能性が高い．この結膜充血は非特異的所見であり，皮疹に先行して結膜炎を発症した場合には，眼科でウイルス性結膜炎と診断されることがあるので注意を要する．

典型的には結膜全体に及ぶ高度な充血，眼瞼の発赤・腫脹，眼脂がみられる．また，偽膜形成に伴って生じる広範囲の角結膜上皮欠損(図3)[2]は本症の特徴的所見であり，細菌あるいはウイルス性結膜炎との鑑別に有用である．

急性期に広範な角結膜上皮欠損を生じた場合には，著しく視力予後不良となる可能性がある．すなわち，急性期に角膜上皮幹細胞が消失すると，上皮欠損部は角膜上皮により修復されず，周囲から伸展する結膜組織で被覆され重篤な視力障害をきたす．一方，角膜上皮欠損を生じても角膜上皮幹細胞が残存した場合には，上皮欠損は角膜上皮により修復され，角膜はほぼ透明化する．急性期の十分な消炎は，角膜上皮幹細胞の残存を可能にする．このため，発症時に眼合併症の有無を検討し，ただちに治療を開始することが患者の視力予後を決定する．ただし，角膜上皮が広範囲に欠損しているときの視力は比較的良好であり，急性期の患者の視力は重症度の指標とはなり得ない．必ず眼科専門医による診察が必要である．

急性期の治療ではステロイドの大量全身投与(ステロイドパルス療法)と眼局所のベタメタゾン投与が有効である．治療が奏効して皮疹が順調に軽快したにもかかわらず，眼表面の炎症が遷延することがある．そのような場合は，皮膚

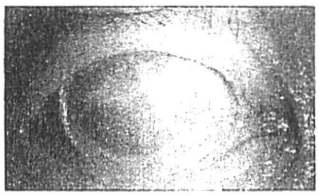


図1　慢性期SJS TENの眼所見
SJS/TENでは重篤な視力障害が後遺症として残ることが多い.

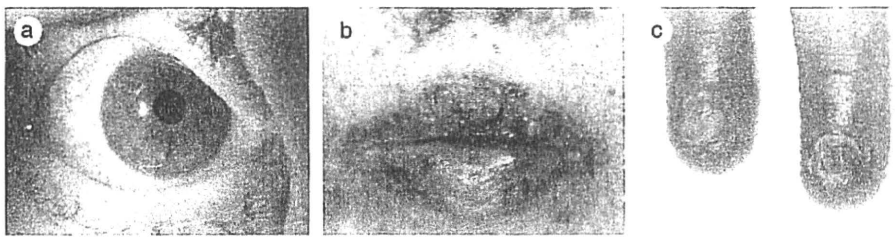


図2　眼合併症を伴うSJS TENの早期診断に有用な所見
眼合併症を伴うSJS/TEN患者では，急性結膜炎(a)，口唇・口腔内の出血性びらん(b)，爪囲炎(c)を必発する.　(文献3より引用)

Essence

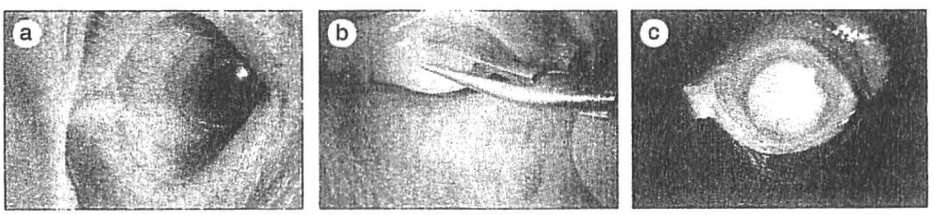


図3　急性期SJS TENの特徴的眼所見
SJS/TEN急性期の結膜炎(a)では，偽膜形成(b：せっしで上眼瞼結膜の偽膜を除去している)と広範囲の角結膜上皮欠損(c：白色の部が角膜上皮欠損部)が認められる.

所見だけではなく眼所見も考慮して，ステロイドの減量を行う．眼局所には，ベタメタゾンの点眼(1日6～8回程度)ならびに眼軟膏(1日4～6回程度)が消炎のために効果的である．角結膜上皮欠損の改善を得ることができれば，ゆっくりと全身と局所のステロイド量を減量していく．ただし，ステロイド投与による感染症発症のリスクがあり，関連する各診療科が連携して感染症(敗血症，肺炎など)の有無を判断して治療を行う．眼表面においても感染症に十分注意して抗菌薬を併用するが，抗菌薬の点眼にもかかわらず，MRSAならびにMRSE感染症を生じることがあるので注意を要する.

Why important?

SJS/TENは生命を脅かすのみでなく，著しい視力障害が後遺症となることを知っておきたい．本症発症による突然の失明は患者の人生を大きく変えてしまう．筆者らが，発症4日以内にステロイドパルス療法と眼局所ベタメタゾン投与を行ったSJS/TEN 5症例では，慢性期に10眼すべてが視力1.0以上であり，角膜上皮幹細胞の指標であるpalisades of vogt (POV)を維持した[5]．急性期の全身状態が重篤であるほど眼には関心が行きにくいが，発症初期より適切な眼科治療を行うことが重要である.

References
1) Yetiv JZ, Bianchine JR, Owen JA Jr : *South Med J* **73** : 599-602, 1980
2) 外園千恵：最新皮膚科学体系 2008-2009 (玉置邦彦 編)．中山書店，東京，2008, p182-188
3) Sotozono C, Ueta M, Koizumi N et al : *Ophthalmology* **116** : 685-690, 2009
4) Ueta M, Sotozono C, Inatomi T et al : *Br J Ophthalmol* **91** : 962-965, 2007
5) Araki Y, Sotozono C, Inatomi T et al : *Am J Ophthalmol* **147** : 1004-1011, 2009

Stevens-Johnson 症候群

Stevens-Johnson 症候群とは

Stevens-Johnson 症候群（Stevens-Johnson syndrome；SJS）および中毒性表皮壊死症（toxic epidermal necrolysis；TEN）は，重症度は異なるが同じスペクトラムに属する急性の全身性皮膚粘膜疾患であり，突然の高熱とともに全身の皮膚・粘膜にびらんと水疱を生ずる．小児を含めてあらゆる年齢に性差なく発症し，発症頻度は人口百万人あたり2～6人/年とされる．

多くが薬剤投与後に発症しており，重篤薬疹[*1]として位置づけられる．誘因となる薬剤は多岐にわたり，抗菌薬，解熱鎮痛薬（NSAIDs；non-steroid anti-inflammatory drugs），抗けいれん薬が代表的な薬剤である．発症機序の詳細は不明であるが，発症に先立ち倦怠感，咽頭痛などの感冒様症状を自覚している症例が多いことから，何らかのウィルス感染が契機になると考えられている．一部の症例；特に小児では *Mycoplasma pneumoniae* との関連が指摘されている．

水疱・びらんが体表面積の10％未満がSJS，10％以上がTENとされる[*2]が，SJSとTENの眼所見は類似しており，眼所見のみで両者を鑑別することは困難である．このため眼科では，SJSとTENを包括してSJSと呼ぶことが多い．

致死率が高いために急性期は全身的治療が主体となるが，しばしば角膜混濁，ドライアイ，睫毛乱生などが生涯にわたる後遺症となる．本症候群の治療にあたっては眼科的病態をよく知ったうえで，発症初期より適切な眼科治療を行うことが重要である．

(*1) 重篤薬疹とは，皮膚以外の臓器に重篤な症状を発現する薬疹であり，生命を脅かしうるものである．SJS，TEN，薬剤性過敏症症候群（drug-induced hypersensitivity syndrome；DIHS）が代表的疾患であるが，このうちDIHSは眼合併症を伴わない．

(*2) SJS班研究の概要のウェブサイト参照．『難病情報センター／皮膚・結合組織疾患：重症多形滲出性紅斑に関する調査研究』
http://www.nanbyou.or.jp/kenkyuhan/rinsyo/38.hifu.htm

急性期の眼障害

両眼の充血，異物感，眼痛が，皮膚病変とほぼ同時に，あるいは皮膚病変より半日から数日先行して生じる[1]．皮疹より眼症状が先行する場合には，眼科においてウイルス性結膜炎あるいは単なる急性結膜炎と診断されることがあり，注意を要する．

文献は p.280 参照.

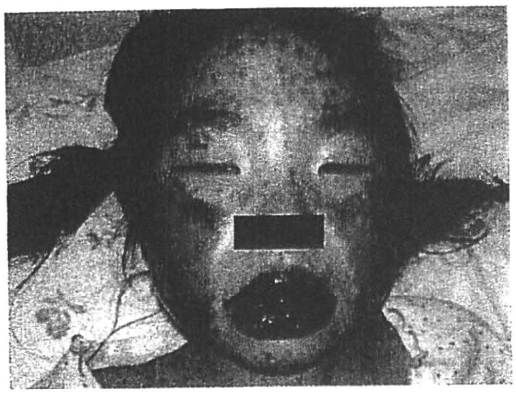
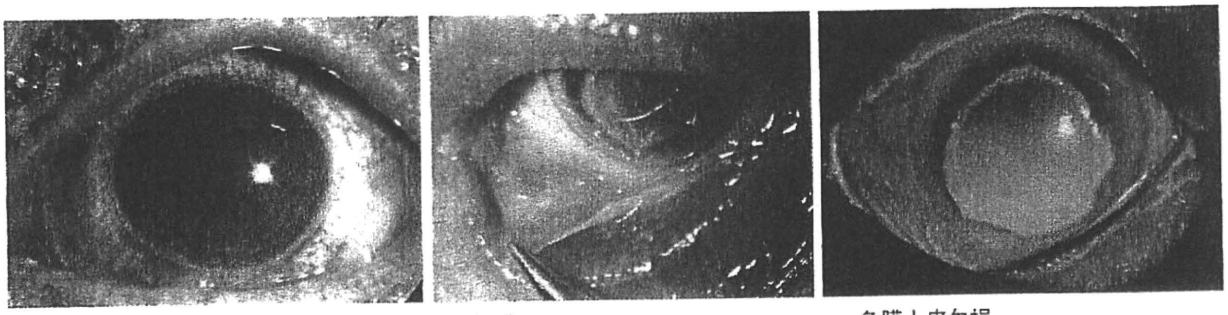


図 1　Stevens-Johnson 症候群
(9 歳，女児)



a. 結膜充血　　b. 偽膜　　c. 角膜上皮欠損

図 2　急性期の眼所見

皮疹は時間単位で急速に広がり，全身の皮膚および粘膜に発疹とびらんを呈する（図 1）．同時に眼病変も急速に進行し，眼瞼の発赤腫脹，結膜全体に及ぶ高度な充血（図 2a），偽膜（図 2b）[*3] を生ずる．さらに広範囲の角膜上皮欠損（図 2c），あるいは結膜上皮欠損を認めれば，眼科的予後がきわめて重篤になる可能性が高い[2]．

急性期に広範な角結膜上皮欠損を生じ，輪部に存在すると考えられている角膜上皮幹細胞がすべて消失すると，上皮欠損部は角膜上皮により修復されずに，次第に周囲から伸展する結膜組織で被覆される[*4]．このため角膜は厚い不透明組織で覆われて表層性の角膜混濁と血管侵入を来たす．一方，上皮欠損を生じても角膜上皮幹細胞が残存した場合には，上皮欠損は角膜上皮により修復され，角膜はほぼ透明化する．

眼合併症を伴う頻度は 70％ 程度とされるが，発疹を生じた時点で両眼が充血している場合は，眼合併症を伴う可能性が高い．また高熱を伴う全身性の薬疹で，口唇・口腔内の発赤・びらん（図 1），爪囲炎（図 3），結膜充血を伴えば，眼合併症を伴う SJS あるいは TEN である可能性が高い[1]．

(*3) 偽膜はフィブリン，壊死上皮細胞、浸潤細胞からなる膜様物である．

(*4) 角膜上皮が広範囲に欠損しているときの視力は比較的良好であり，急性期の患者の視力は重症度の指標とならない．

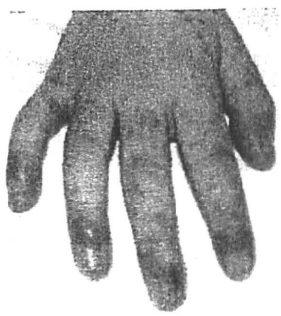


図 3　爪囲炎

急性期の治療

急性期の十分な消炎は，角膜上皮幹細胞の残存を可能にし，視力予後を良好にする．眼合併症を可能な限り防ぐには，発症初期より，眼表面の炎症を抑えて眼表面上皮を温存し，同時に眼表面の二次感染防止を行うことが有用である[*5]．

消炎：急性期の治療は，皮膚科との連携が必要であり，発症早期のステロイド大量全身投与（パルス）が有用である．皮疹が順調に軽快しても，しばしば眼表面炎症が遷延するため，ステロイドの減量は皮膚科的所見だけではなく眼科的所見も考慮して行わねばならない[*6]．

眼局所にはベタメタゾンの点眼（1日4～6回程度）を行い，炎症が高度な場合にはベタメタゾン眼軟膏（1日4回程度）を併用する．感染症を招くおそれがあるため，患者と家族に感染症発症のリスクと本症の視力予後について十分に説明したうえで，抗菌薬を併用しながらステロイドを局所投与する．角結膜上皮欠損の改善を得ることができれば，全身と局所のステロイド量をゆっくりと減量する．

感染予防：初診時に眼分泌物の塗抹および培養検査を行い，その後は週1回程度の監視培養を継続する．患者は全身的にも，眼表面にもMRSAあるいはMRCNS[*7]を保菌しやすい．何らかの菌を検出すれば薬剤感受性を考慮して，抗菌薬を局所投与する．

偽膜除去と癒着解除[*8]：生じた偽膜はていねいに除去する．偽膜除去の操作が炎症を惹起してはならず，清潔な綿棒に絡めとるように除去するか，先端が丸い摂子で除去するとよい．

急性期に生じる瞼球癒着を放置すると強固な器質的癒着となる．点眼麻酔下にガラス棒を用いて機械的に癒着を剝離する．

遷延性上皮欠損：selflimitedの疾患であり，通常3～4週間程度で皮膚および全身症状が落ち着いてくるが，眼表面の炎症が依然続いて広範囲の上皮欠損が遷延することがある．遷延性上皮欠損（**図4**）は，突然の角膜感染症，あるいは角膜融解や穿孔を来たすリスクが高い．保存的治療で上皮の修復を得られない場合には，培養粘膜上皮シート移植が有用である[3]．

慢性期の眼障害

上述したように急性期に角膜上皮幹細胞を消失すると，結膜が角膜表面に伸展する．血管と結合織を伴った結膜組織が角膜を覆うた

(*5) 発症時の眼科診察は最低1日1回行い，炎症が高度あるいは変化が著しい場合には，朝夕2回の診察が望ましい．

(*6) 皮膚所見よりも眼所見やほかの粘膜所見の改善が遅い場合に，皮膚所見に応じてステロイド減量を行うと，眼所見の悪化を生じて難治となることがある．

(*7)
MRSA
メチシリン耐性黄色ブドウ球菌
(methicillin-resistant *staphylococcus aureus*)

MRCNS
メチシリン耐性コアグラーゼ陰性ブドウ球菌
(methicillin-resistant coagulase negative staphylococci)

(*8) 偽膜，瞼球癒着は眼表面炎症の指標となり，これらを認める時期には消炎に努める．治療が奏効すると上皮欠損は次第に縮小し，偽膜がみられなくなる．

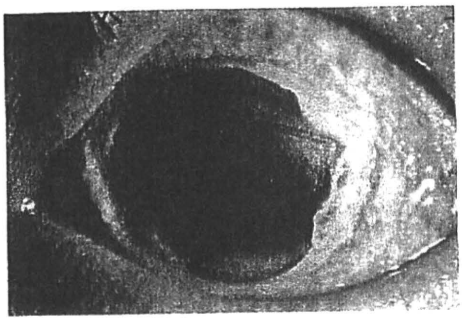


図4　遷延性上皮欠損

29歳，女性．Stevens-Johnson症候群発症後4か月．角膜表面にカルシウム沈着があり，このためフルオレセインで染まらないが，角膜は全上皮欠損である．結膜上皮も広範囲に欠損し，結合組織の肥厚，増殖を認める．

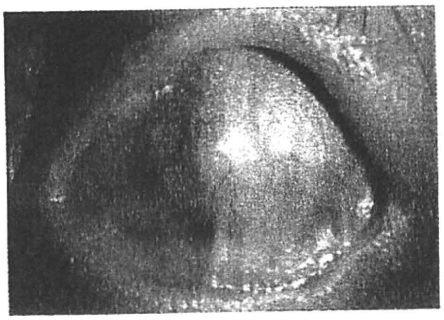


図5　慢性期の眼所見

図4症例の4年後．MRSA角膜感染症，その治癒後に非感染性の角膜穿孔を生じた．最終的に上皮欠損は結膜組織により被覆された．角膜混濁，血管侵入，表面の凹凸不整が著しく，上皮は異常分化により角化している．視力は眼前手動弁であり，高度の涙液分泌不全，睫毛乱生を合併する．

め，角膜表面は不透明，凹凸不整となって視力障害を来たす．重症では上皮が異常分化を示して眼表面が皮膚のように角化し，視力が著しく低下する（図5）[4]．

視力を温存できた場合でも，しばしば涙腺導管の閉塞による涙液分泌不全に加えて，マイボーム腺（meibomian gland）の腺構造消失による蒸発亢進型ドライアイを合併する．高度のドライアイのために，乾燥感，異物感，羞明，眼痛などが眼症状として持続する．

炎症の後遺症として睫毛乱生，瞼球癒着などの瘢痕性変化が存在し，これらも異物感，羞明といった不快な症状の原因となる．

慢性期の治療

慢性期の治療は，人工涙液，睫毛抜去，感染予防などの対症療法，保存的治療が主体となる．

睫毛乱生を放置すると眼表面の炎症や感染症を生じるため，2～4週に1回程度の通院により睫毛を抜去する．多重睫毛を伴う場合には，睫毛根部の切除が有用である．

ドライアイに対しては，涙点プラグまたは涙点縫合を行い，ヒアルロン酸の点眼（4～6回），防腐剤を含まない人工涙液の頻回点眼を行う．

視力回復目的の上皮移植は予後不良とされてきたが，近年は培養粘膜上皮シート移植による視力改善が可能となってきた[5]．

（外園千恵）

本項は，厚生労働省科学研究費補助金（難治性疾患克服研究事業）の援助を受けた．

エビデンスの扉

厚生労働省 SJS 班研究の概要を教えてください

背景

Stevens-Johnson 症候群（Stevens-Johnson syndrome；SJS），その重症型である中毒性表皮壊死症（toxic epidermal necrolysis；TEN）は，いずれも突然の高熱，咽頭痛に続いて全身の皮膚・粘膜にびらんと水疱を生ずる急性の全身性皮膚粘膜疾患である．ほとんどが何らかの薬剤投与を契機に発症しており，重篤な薬剤副作用でもある．しばしば敗血症，播種性血管内凝固（disseminated intravascular coagulation；DIC）症候群，多臓器障害などを併発し，特に TEN では高い死亡率（20％前後）が報告されている．

急性かつ重篤な疾患であることから，速やかな診断と治療の開始が必要とされるが，以下のような問題点を有する．

表 1　Stevens-Johnson 症候群診断基準 2005

1. 概念

発熱を伴う口唇，眼結膜，外陰部などの皮膚粘膜移行部における重症の粘膜疹（図 1）および皮膚の紅斑で，しばしば水疱，表皮剝離などの表皮の壊死性障害（図 2）を認める．原因の多くは薬剤である．

2. 主要所見（必須）

① 皮膚粘膜移行部の重篤な粘膜病変(出血性あるいは充血性）がみられること．
② しばしば認められるびらん，もしくは水疱は体表面積の 10％未満であること．
③ 発熱．

（副所見）

④ 皮疹は非典型的ターゲット状多形紅斑．
⑤ 角膜上皮障害と偽膜形成のどちらか，あるいは両方を伴う両眼性の非特異的結膜炎．
⑥ 病理組織学的に，表皮の壊死性変化を認める．

ただし，TEN への移行がありうるため，初期に評価を行った場合には，極期に再評価を行う．

主要項目の 3 項目をすべて満たす場合，SJS と診断する．

TEN：中毒性表皮壊死症
（厚生労働科学研究費補助金難治性疾患克服研究事業 重症多形滲出性紅斑に関する調査研究班．）

表 2　中毒性表皮壊死症（TEN）診断基準 2005

1. 概念

広範囲な紅斑と，全身の 10％以上の水疱，表皮剝離・びらんなどの顕著な表皮の壊死性障害を認め，高熱と粘膜疹を伴う．原因の大部分は医薬品である．

2. 主要所見（必須）

① 体表面積の 10％を超える水疱，表皮剝離，びらんなどの表皮の壊死性障害．
② ブドウ球菌性熱傷様皮膚症候群(SSSS)を除外できる．
③ 発熱．

3. 副所見

④ 皮疹は広範囲のびまん性紅斑および斑状紅斑である．
⑤ 粘膜疹を伴う．眼症状は眼表面上皮びらんと偽膜のどちらか，あるいは両方を伴う両眼性の非特異的結膜炎．
⑥ 病理組織学的に，顕著な表皮の壊死を認める．

主要 3 項目のすべてを満たすものを TEN とする．

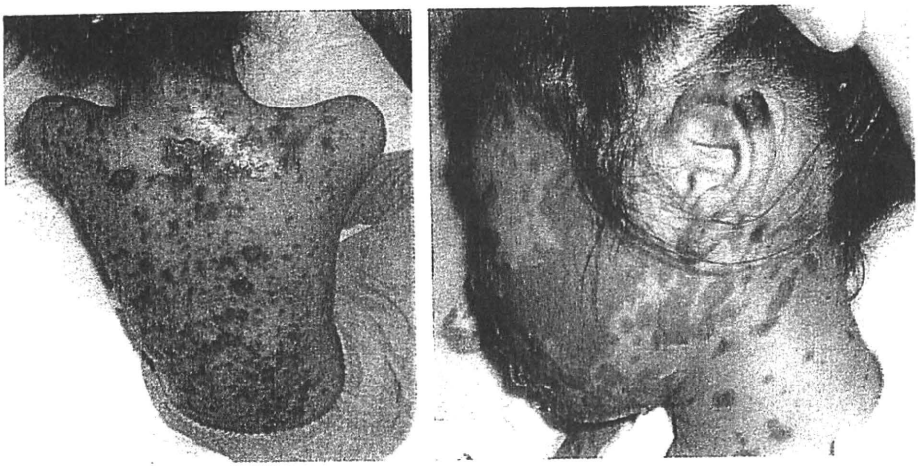
サブタイプの分類

1 型：SJS 進展型（TEN with spots）
2 型：びまん性紅斑進展型（TEN without spots）
3 型：特殊型

参考所見

治療等の修飾により，主要項目 1 の体表面積 10％に達しなかったものを不全型とする．

SSSS：staphylococcal scalded skin syndrome
（厚生労働科学研究費補助金難治性疾患克服研究事業 重症多形滲出性紅斑に関する調査研究班．）



a. 背中　　b. 顔から頸部

図1　SJS による皮疹
（9 歳，女児）

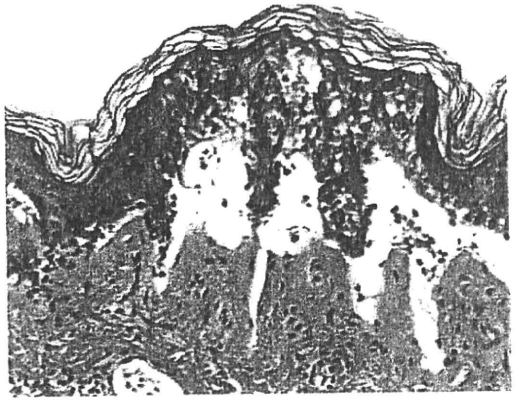


図2　表皮の壊死性変化

1. 国際的に定まった診断基準がない．
2. ステロイド投与の可否について国際的に賛否両論があり，治療指針がない．
3. 視力障害，呼吸器障害などの後遺症が重篤である．

古くから SJS と重症多形滲出性紅斑（erythema exsudativum multiforme major；EEMM）は同一疾患と考えられてきたが，1985～1995 年にかけて大規模な international prospective study が行われ，SJS と TEN は重症度の異なる同一スペクトラムの疾患であり，EEMM とは異なるという考えが主流となった[1]．しかし実際の臨床現場では，EEMM やほかの重症薬疹である薬剤性過敏症症候群（drug-induced hypersensitivity syndrome；DIHS）との鑑別など，診断や治療に苦慮することが少なくない．特に，初期治療や進行時の治療の目安となる指針の作成が求められるようになった．

文献は p.280 参照.

そこで，平成 17（2005）年度に厚生労働科学研究補助金難治性疾患克服研究事業の重症多形滲出性紅斑に関する調査研究班により SJS および TEN の診断基準が作成され（表 1, 2），引き続き平成 18（2006）年度に治療指針案が示された[2]．これに若干の修正を加えた