皮膚粘膜眼症候群の眼後遺症

外園千恵[*1]　上田真由美[*2]　木下　茂[*3]

Stevens-Johnson 症候群（SJS）は皮膚粘膜眼症候群と呼ばれてきた疾患であるが，近年は重症薬疹，すなわち「臓器障害を伴い，死に至りうる薬疹」として分類される．発疹が体表面積の 10% 未満が SJS，10% 以上に及ぶと中毒性表皮壊死症（TEN）とされる[1]．いずれも高熱を伴って突然に発症し，全身性の発疹に加えて粘膜の水疱，びらんを伴う．あらゆる薬剤が発症契機となることから，すべての診療科の医師が遭遇しうる疾患である．

SJS と TEN の急性期は，救命が治療の主目的となる．しかし，全身状態が回復した後に著しい視力障害に陥ることが少なくない．典型例では眼瞼と眼球が癒着（瞼球癒着）し，角膜は結膜組織で覆われて混濁する[2]（図 1）．高度ドライアイを伴い，「見えない，痛い，乾く」という症状が生涯に及ぶ後遺症となる．

眼科患者を対象としたわれわれの調査では，患者の約 8 割が皮膚粘膜症状を生ずる前に全身倦怠感，咽頭痛といった感冒様症状を自覚し，これに対して総合感冒薬，抗菌薬，解熱鎮痛薬等を服用していた．その後，眼の痛みや充血の自覚があり，発症時にはほぼ全例が口腔内・口唇周囲に出血性の水疱・びらんを伴った[3]．したがって，感冒様症状に対して何らかの薬剤を服用した後に，38℃ 以上の高熱と全身性の発疹を生じ，眼の充血，口腔内・口唇周囲に出血性の水疱・びらんを伴う場合は重症薬疹の可能性が高い．

軽い充血に見えても広範囲に角結膜の上皮欠損を伴うことがあり，肉眼での判断は危険である．痛くて眼を開けられないという症状は要注意であり，眼表面に著しい炎症が存在する．広範囲の上皮欠損と著しい炎症は上皮の幹細胞疲弊を招き，次第に眼表面の癒着と瘢痕化が進行する．

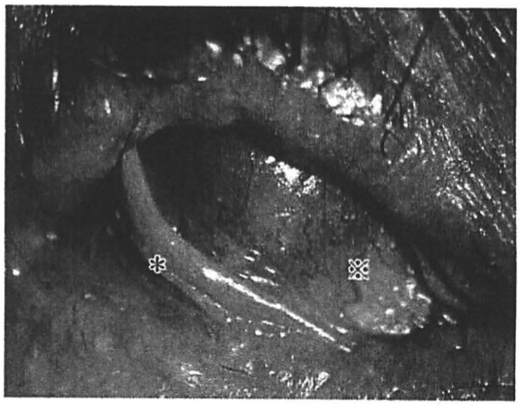

図 1　Stevens-Johnson 症候群の眼後遺症
急性期に広範囲の上皮欠損を生じた後に周囲の結膜が侵入し角膜混濁を来す．眼球と眼瞼の癒着があり（*），鼻側の上皮は角化している（※）．

厚生労働省研究班では 2005 年に診断基準を作成し，医薬品医療機器総合機構ホームページに主な症状と治療指針を提示した[4,5]．研究班ではステロイドパルス療法を提唱しており，眼科的にもベタメタゾン点眼等を用いて，眼表面を徹底して消炎することが上皮欠損や幹細胞疲弊の回避につながる．ただし，ステロイド投与は感染症を招くリスクがあるため，ウイルス感染，MRSA の保菌等をチェックしてから全身と眼表面の消炎を行う．

SJS，TEN の発症による中途失明は患者の人生を大きく変えてしまう．発症時のすみやかな診断と，発症初期から眼科医が治療チームに加わり，適切な眼科治療を行うことが重要である．

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角　膜　各シーズの臨床研究進捗状況

培養粘膜上皮移植の長期成績*1

Longterm outcomes of cultivated oral mucosal epithelial transplantation

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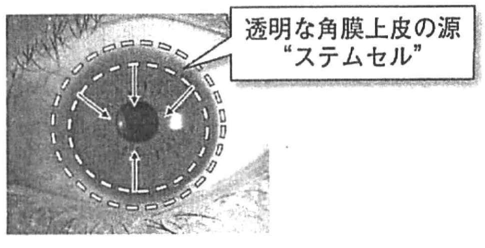
1. 眼球の構造と難治性疾患

簡単ですが，目の構造のイントロダクションをさせていただきます.

眼球はカメラに例えられ，表から光が入って角膜，水晶体を通って網膜に外界が投影されます．我々が扱っているのは表側の角膜で，角膜の中でも最表層の上皮が濁ると，眼球内たとえば水晶体や網膜が健常であっても見えなくなってしまいます．角膜上皮のステムセルは，角膜の周辺部の点線で取り囲まれたドーナツ型の輪部と呼ばれるところに存在しています (Fig. 1)．重症薬疹として発症するStevens-Johnson症候群，化学外傷・熱傷などで角膜上皮のステムセルが消失すると，Fig. 2のように周辺から混濁した組織である結膜が侵入して，ほとんど見えない状態になってしまいます．自己免疫疾患である眼類天疱瘡という疾患では，次第に眼球と眼瞼（まぶた）の癒着が進み，眼表面が先の2疾患と同じような状態になってしまいます．角膜疾患の中でも，このように角膜上皮のステムセルが疲弊したものは非常に難治で，重症であるにもかかわらず，従来の角膜移植では視力を維持することができず，治療できない疾患として長く扱われてきました.

この難治性疾患に対して，何とか治療できないかということで，我々のチームで長く研究に取り組んでいます.

Fig. 1　正常な角膜上皮の供給



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2. 培養粘膜上皮移植の経緯と現状

我々が初めて培養上皮シート移植を開始したのは1999年であり，10年以上継続しております．培養細胞を用いた実験，ウサギの実験を経て倫理委員会の承認のもとに培養角膜上皮シート移植を開始しました[1～6]．これはドナーの健常な輪部からとったステムセルを体外で培養し，シート状にして角膜上皮に類似したものをつくり，患者の角膜を覆う混濁した上皮をとって移植するというもので，成功して当時はマスコミでも話題になりました（Fig. 3）．

これはアロとオートと2通りできますが，両眼性の疾患の場合にはオートの移植ができません．そこで両眼性の難治性疾患を対象に自家の細胞移植として培養口腔粘膜上皮移植を2002年から開始しています[7～11]．今後どう実用化につなげる

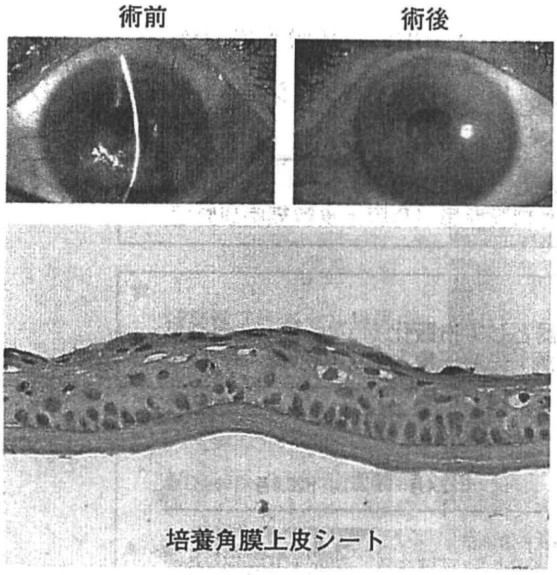
Fig. 2　難治性角結膜疾患（角膜上皮幹細胞疲弊症）

- 角膜上皮幹細胞が広範囲に障害
- 角膜上への結膜侵入による重度の視力障害
- 通常の角膜移植では再建困難

視力回復による社会復帰をめざす

Fig. 3　背景と目的

- 1999年より培養角膜上皮シート移植を開始
- 2002年より培養口腔粘膜上皮移植を開始
- 研究成果を実用化に繋げることを目的とする



Ophthalmology. 108:1569, 2001


羊膜上で角膜培養，そのまま移植
京都府立医大 新手法を開発
広範囲の障害に効果
遺伝子治療への応用も

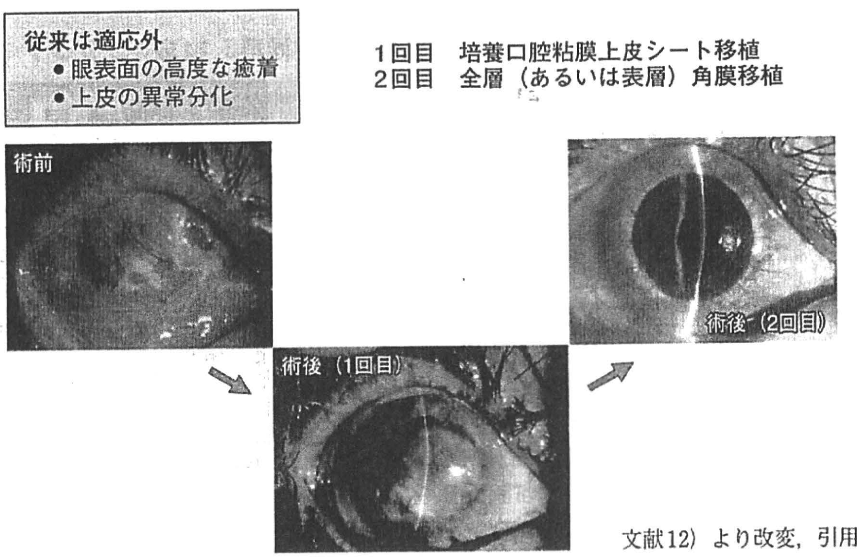
2000年7月17日　共同通信など

めに，手術前は人に手を引いてもらうか，白杖を持って前に進むという状態の方がほとんどです．手術で1.0以上の視力を得るのは難しいですが，0.01あるいは0.02以上が出れば，自分で身の回りのことができるようになります．病気になったことで人生が変わった患者も少なくありませんが，この手術をすることで，明るい展望を開くことができると考えています．

同じような疾患でも，眼表面の上皮細胞が異常分化を起こして「角化」と呼ばれる，皮膚のような状態になった場合には，どのような手術をしても治せないとされてきました．しかし，培養口腔粘膜上皮移植により眼表面の上皮を取り換えて，次に全層角膜移植，あるいは表層角膜移植という従来型の移植を行うことで，回復の見込みのなかった症例でも希望を見出すことができるようになりました（Fig. 8）[12]．現在までに，大まかに分けて3つの病態に対して手術を行っており，視力改善を目的とした手術を52眼，結膜嚢再建という眼球と眼瞼の癒着を外すという手術を26眼に行い，急性期に化学外傷を受けて上皮が治らないなど遷延性上皮欠損における上皮修復目的でも手術を行いました（Fig. 9）[13]．

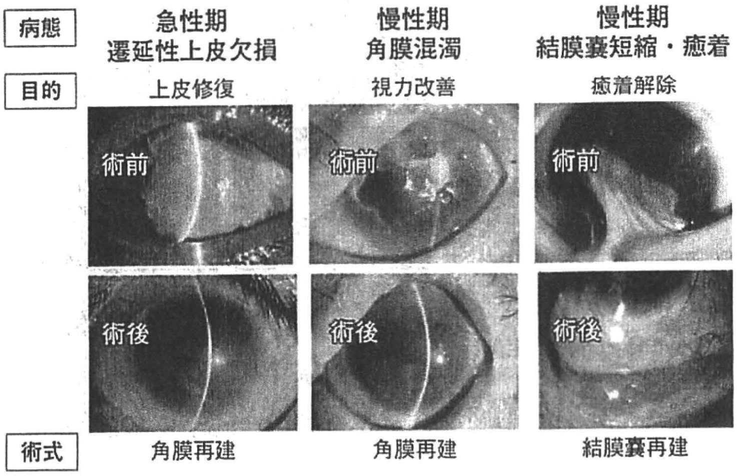
これらの解析では視力以外に眼表面の病的状態

Fig. 8　難易度の高い症例に対する新しい手術方法



文献12）より改変，引用

Fig. 9　先行臨床試験



を評価する客観的スコアを決めており，角膜再建患者の術前スコアを1とした場合に術後の平均が約0.4となっております（Fig. 10）．術後半年の経過後は長く効果を維持し，視力改善も100％ではないものの2段階以上の視力改善を7割で得ている状況です．癒着解除においても，6カ月以上長期に効果が維持しており（Fig. 11），もともと治せないことを考えると，満足度は高いと思っています．

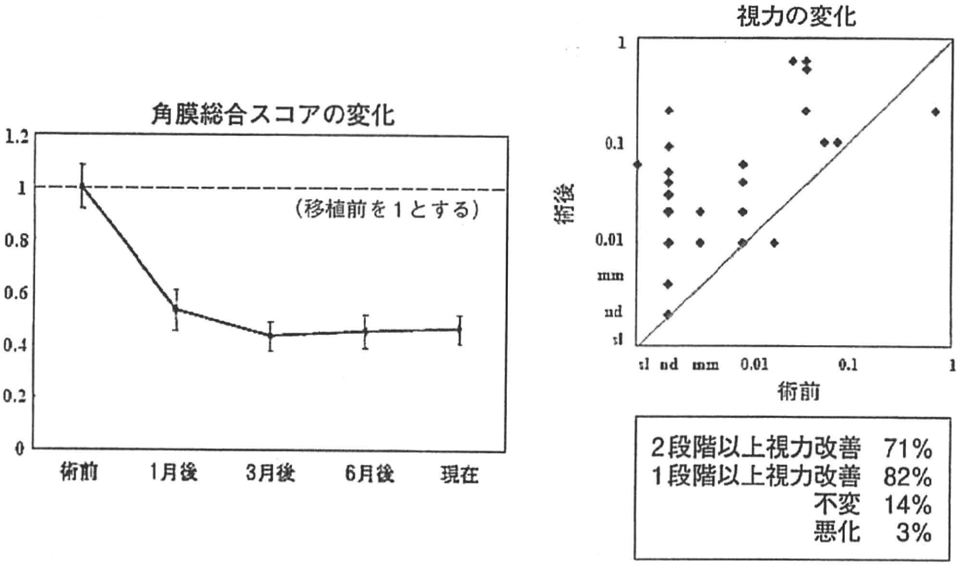
5. 今後の課題

これまでに医師が行ってきた臨床研究あるいは基礎研究とは異なり，細胞を使った治療をどうやって実用化していくかということが現在の課題となっています．再生医療の実用化に関する種々の問題は，我々の一施設だけではとても解決できません．安全性を担保したものをどうやって臨床現場に届けるかということを話し合い，進めている段階です．さらには手術適応を明確化し，術後治療の標準化も行って，広く社会で実施していただける有意義な治療にしたいと考えております．

付　記

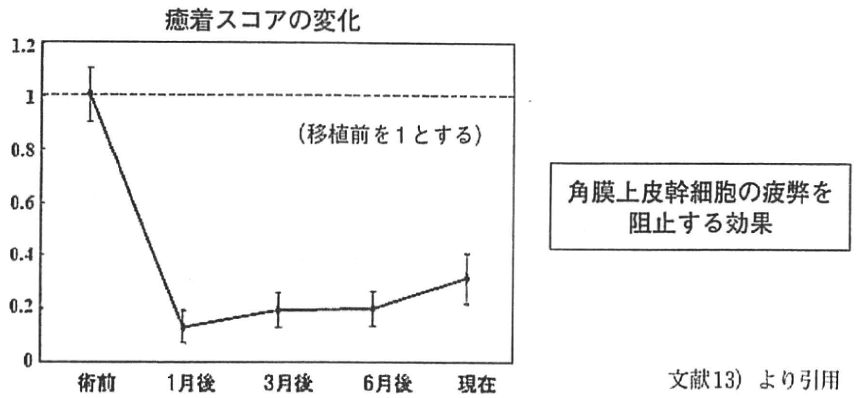
本論文は厚生労働科学研究費補助金（難治性疾患克服研究事業）(H22-難治--一般-003）の援助を受けた．

Fig. 10　慢性期角膜再建　52眼



文献13）より改変，引用

Fig. 11　結膜嚢再建　26眼



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* * *

Immune Processes and Pathogenic Fibrosis in Ocular Chronic Graft-Versus-Host Disease and Clinical Manifestations after Allogeneic Hematopoietic Stem Cell Transplantation

Yoko Ogawa, MD, Shigeto Shimmura, MD, Murat Dogru, MD, and Kazuo Tsubota, MD

Abstract: Activation of the immune system and progressive fibrosis are prominent features of chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation. The main histologic findings in affected exocrine glands are marked fibrosis of the interstitium and prominent increase in the number of fibroblasts, accompanied by mild lymphocytic infiltration. Clinically, the severity of dry eye is correlated with the degree of fibrotic changes, rather than with the amount of lymphocytic infiltration, indicating that excessive extracellular matrix accumulation primarily contributes to the exocrine dysfunction. We have found that accumulated fibroblasts in the lacrimal gland of patients with cGVHD have a chimeric status. Thus, fibroblasts originating from circulating donor-derived precursors and recipient-derived fibroblasts by local epithelial mesenchymal transition may participate in the excessive fibrosis in patients with cGVHD by interacting with T cells. In this article, the immune response and pathogenic fibrosis in ocular cGVHD will be discussed. In addition, clinical findings of ocular complications after hematopoietic stem cell transplantation and currently available treatment will also be discussed.

Key Words: hematopoietic stem cell transplantation (HSCT), chronic graft-versus-host disease (cGVHD), dry eye, pathogenic fibrosis, immune response

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a technique that was originally developed by E. Donnall Thomas who was honored for endeavors in experimental and clinical bone marrow transplantation by receiving the Nobel Prize for Physiology or Medicine in 1990. Now, HSCT is a potentially curative treatment for a number of hematological malignancies and genetic disorders; worldwide, >25,000 HSCT procedures are performed annually and the number is increasing.[1]

Chronic graft-versus-host disease (cGVHD) is a major late complication after allogeneic HSCT and has been a strong barrier against successful outcome in this setting.[1] On the other hand, the occurrence of graft-versus-host disease (GVHD) in leukemia also reduces the risk of relapse—the graft-versus-leukemia (GVL) effect. Therefore, a balance between GVHD severity and induction of GVL is desirable. Likewise, topical treatment of ocular manifestations of GVHD exerts benefit without reduction of GVL when systemic immunosuppressant was tapered.

Recently, there have been many advances in the treatment of acute GVHD (aGVHD). Moreover, understanding of the pathogenesis of cGVHD has gradually progressed.[2] Because of wide application of HSCT for pediatric[3,4] and aged populations, thorough understanding of the pathogenesis underlying cGVHD and its prophylaxis and treatment are critical issues that deeply affect transplant recipients' quality of life.

BASIC RESEARCH ON OCULAR cGVHD

Histologic studies of cGVHD have suggested some immunological targets and immune-mediated fibrosis.[5] Fibroblasts play a central role to develop fibrosis in affected organs. Fibroblasts are not merely components responsible for making the body structure but also may be involved in the immune response of several diseases including scleroderma,[6] rheumatoid arthritis,[7] and Graves disease[8] as well as GVHD.[9]

We have examined the pathogenesis of cGVHD by focusing on the lacrimal gland—one of the most frequently affected organs in patients with cGVHD. We found an increased number of CD34^{+} fibroblasts and excessive fibrosis, indicating that extracellular matrix (ECM) accumulation contributes to exocrine dysfunction in this context.[10] Fibroblasts also play a role in inflammation where they attach to lymphocytes and express histocompatibility class II and costimulatory molecules. Such fibroblasts were not observed in Sjögren syndrome (SS) lacrimal glands and normal conjunctiva, suggesting that a unique subpopulation of fibroblasts contributes to the immune processes and pathogenic fibrosis in ocular cGVHD.[11]

Recent evidence suggests that engraftment of non-hematopoietic cells occurs in various organs of HSCT

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recipients.[12,13] In addition, recent findings in animal models indicate that a significant proportion of mesenchymal cells involved in tissue injury may be derived from bone marrow or from circulating fibrocytes.[14,15] These reports and our previous findings on the unique phenotype of fibroblasts that accumulate in cGVHD lesions led us to hypothesize that a subset of these cells originates from the transplanted donor graft. In pathogenic fibrotic areas, we detected donor fibroblast chimerism in cGVHD lacrimal gland. By detecting mismatched genetic markers in tissue specimens and primary fibroblast cultures, we determined that nearly half the CD34⁻ fibroblasts at the site of pathogenic fibrosis were of donor origin (Fig. 1).[16] Donor-derived conjunctival epithelia and myofibroblasts have also been detected after sex-mismatched HSCT by staining for the Y chromosome in female allograft recipients.[17,18] However, the proportion of such cells is very small, and their contribution to pathogenic processes remains unknown.

On the other hand, several studies have reported that epithelial–mesenchymal transition (EMT) contributes to various fibrotic diseases of the kidney, lung, and liver as well as the eye.[19,20] For example, in kidney fibrosis, 40% of fibroblasts arise from epithelial cells via local EMT.[21]

EMT is characterized by loss of cell–cell adhesion and apical/basal cell polarity followed by acquisition of a mesenchymal phenotype and migration and invasion capabilities.[19] EMT is triggered by various stimuli including irradiation,[22] hypoxia,[23] reactive oxygen species,[24] inflammatory cytokines such as transforming growth factor (TGF) β and fibroblast growth factor,[19] and disruption of basal lamina and exposure of cytoplasm to ECM.[25] There are various stimuli in HSCT recipients that can trigger EMT. Clinically, total body irradiation performed before HSCT and migration of inflammatory cells thereafter lead to the generation of large amounts of proinflammatory cytokines.[26] This "cytokine storm" acts on T cells in the graft, prompting them to attack host antigens. In addition, reactive oxygen species–mediated organ injury has been reported in bone marrow transplantation recipients.[27] We therefore analyzed whether EMT may be involved in the mucosal and exocrine glands in patients with cGVHD.[28]

We studied 13 allogeneic HSCT recipients who had dry eye associated with cGVHD. Lacrimal gland and conjunctival biopsies from patients with cGVHD were taken for diagnostic purposes in all patients. Biopsy specimens were compared with normal conjunctival tissue samples and lacrimal gland samples from patients with SS serving as controls. This study

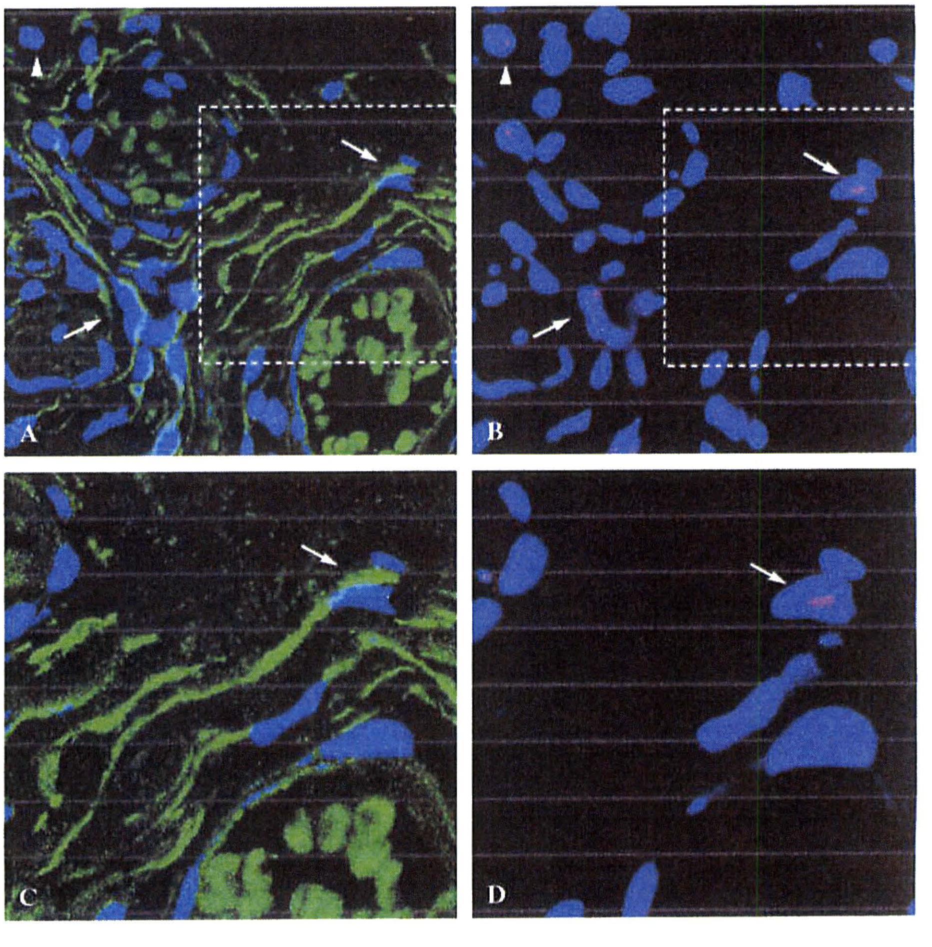

FIGURE 1. Representative CD34 immunostaining and Y chromosome fluorescein in situ hybridization (Y-FISH) images of the same lacrimal gland section from a female patient with cGVHD after HSCT from a male donor. The left panels show CD34 staining (green, A, C) and right panels Y-FISH (red, B, D) with TO-PRO-3 staining (blue) of the same region. C, D, Higher magnification views of lesions shown in dotted squares. Arrows indicate CD34⁺ fibroblasts with Y-FISH signal; arrowheads indicate Y-FISH⁺ cells negative for CD34. Red blood cells in the vascular lumen emit nonspecific autofluorescence. Reprinted with permission from Ogawa et al.[16]

was approved by Keio University Institutional Review Board. By analyzing tissue sections, Mallory staining revealed severely fibrotic subepithelial stroma with mild lymphocytic infiltration in cGVHD specimens. The cGVHD epithelium was thin, and the basal lamina was attenuated compared with normal conjunctiva and SS lacrimal gland.

Next, to examine the expression pattern of EMT markers, we looked at E-cadherin, β-catenin, and Snail.[29] In normal conjunctiva, a characteristic pattern of E-cadherin staining was seen with labeling at the intercellular junctions of all epithelial layers as well as characteristic membranous pattern of β-catenin. In contrast, in GVHD epithelia, intercellular staining of E-cadherin was diminished, and β-catenin was expressed in the cytoplasm and the nucleus. Snail, an inducer of EMT and transcriptional repressor of E-cadherin, was also expressed in the nucleus of cGVHD epithelial cells but not in normal conjunctival epithelia. This observation is consistent with repression of E-cadherin expression by Snail.

Electron microscopy revealed that the conjunctival epithelia of patients with cGVHD consists of only 2–3 cell layers; cell–cell adhesion between basal cells is disrupted, with dissociated basal epithelial cells having spindle-shaped morphology and cytoplasmic extensions containing ECM in the cytoplasm. Abnormal collagen bundles with unusual periodicity were present in the subepithelial stroma (Fig. 2A, B).[28] The cGVHD conjunctiva showed attenuated and elongated processes extending into the subconjunctival stroma through the disrupted basal lamina. Fibroblasts situated therein showed features of activation including large amounts of protein in the rough endoplasmic reticulum (Fig. 2C, D).[28]

To examine epithelial plasticity to mesenchymal phenotype in cGVHD, we assessed mesenchymal markers such as α–smooth muscle actin and heat shock protein 47 (HSP47) on cGVHD epithelia. Both these proteins were expressed in basal epithelium of cGVHD conjunctiva but not in control tissue. Moreover, electron microscopy revealed abnormal collagen bundles in the cytoplasm of basal cells at basement membrane–disrupted sites in cGVHD samples but not in normal controls. These findings seem to imply that ocular cGVHD epithelium gains mesenchymal phenotype. Whereas basement membrane plays a role in preventing EMT, this can occur in its locally disrupted sites.[30]

We next examined the expression of p63, an epithelium-specific transcription factor that is synthesized predominantly in basal cells of a variety of epithelia.[31] Whereas p63 was located in the nucleus of basal cells in normal conjunctiva, this protein was found in some cellular nuclei in the conjunctival stroma in cGVHD samples (Figs. 3A–C).[28] Type IV collagen staining revealed disruption of basal lamina in cGVHD conjunctiva (Fig. 3D), whereas that in normal conjunctiva was continuous (Fig. 3E). The average area of disrupted basal lamina was statistically greater in GVHD conjunctiva than in normal controls (Fig. 3F). Double staining for p63 and type IV collagen revealed that $p63^+$ stromal cells were preferentially located below the type IV collagen layer in regions where this layer showed degradation, probably because of epithelial cell plasticity and acquisition of cell invasion capabilities (Figs. 3G, H).

Double staining for nuclear p63 and cytoplasmic HSP47 revealed expression of both these proteins among basal cells and subconjunctival cells obtained from patients with cGVHD (Figs. 3I–K). In contrast, no double-positive cells were detected in the basal or stromal cells from normal conjunctiva (Fig. 3L). MMP9, type IV collagenase, was expressed in cGVHD conjunctival epithelia (Fig. 3M) but not in normal conjunctiva (Fig. 3N). The number of p63/HSP47 double-positive invading cells in the cGVHD conjunctival samples was significantly higher than that in normal samples (Fig. 3O).[28] These findings suggest that some basal epithelial cells migrated into the conjunctival stroma through disrupted basal lamina after digestion by MMP9.

Similar findings were obtained in lacrimal gland myoepithelia. In exocrine organs, stellate myoepithelial cells are observed between the basal lamina and acinar or ductal cells (Fig. 4). These cells have structural features of both epithelium and smooth muscle cells. Their functions include contraction when the gland is stimulated to secrete, compressing or reinforcing the underlying parenchymal cells, and preventing damage to surrounding cells.[32] EMT signaling pathways alter the stability of junctional complexes by destabilizing the cortical cytoskeleton.[19]

As biomarkers of EMT, abundant intermediate filaments and microfilaments, gain of rough endoplasmic reticulum, abundant lysosomal granules, and loss of intercellular junctions on electron microscopy have been reported.[33] In cGVHD lacrimal gland myoepithelia, electron microscopy revealed remarkable changes in the microfilament cytoskeleton and elongation of cytoplasmic processes from cGVHD lacrimal gland myoepithelia rich in actin filament along the apicobasal axis into the subepithelial stroma (Fig. 4A, B) compared with normal lacrimal gland myoepithelia (Fig. 4E). Furthermore, abundant collagen bundles were seen adjacent to the myoepithelium (Figs. 4C, D).[28] Together, these findings indicate that cGVHD fibroblasts may be partially derived from the epithelia via EMT.

Based on previous reports and our findings, we propose the following model for the pathogenic process of cGVHD. Donor fibroblasts expressing human leukocyte antigen class II and costimulatory molecules act as antigen-presenting cells that generate a cytokine storm and trigger cGVHD. Migrating donor fibroblasts may interact and collaborate with T cells and recipient-derived fibroblasts and thereby contribute to cGVHD pathogenesis.[34]

A murine model of lacrimal gland involvement in GVHD has shown features that closely mimic those observed in humans.[35] We are still seeking to determine the precise mechanisms underlying the pathogenesis of cGVHD using another animal model that resembles human ocular cGVHD.[36] In particular, it is hoped that our research may help to elucidate the pathogenesis of cGVHD fibrosis and facilitate development of novel antifibrotic therapies.

CLINICAL MANIFESTATIONS OF OCULAR cGVHD AND OTHER COMPLICATIONS AFTER HSCT

Dry eye has been recognized as a complication and one of the distinctive features of cGVHD. Recently, new diagnostic criteria for cGVHD have been proposed; these as well as tools for diagnosis and scoring of cGVHD will be updated

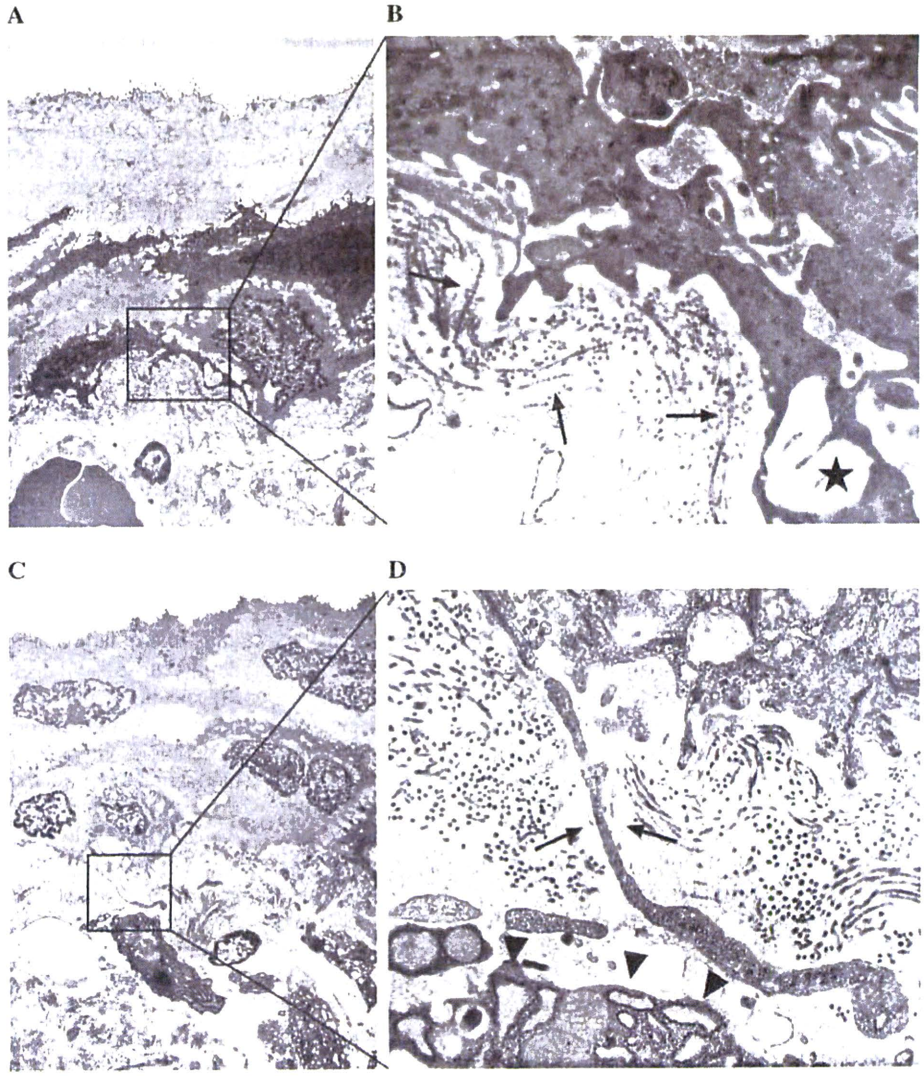

FIGURE 2. Electron microscopic images of disrupted basal lamina and epithelial cells in cGVHD. A, Conjunctival epithelia in cGVHD comprising only 2–3 cell layers, with dissociated cell–cell adhesion among basal cells. Magnified view of the boxed area (B) revealing that the dissociated basal epithelium has spindle-shaped morphology with cytoplasmic extensions containing ECM (star) in the cytoplasm. Abnormal collagen bundles with unusual periodicity (arrows) were present in the subepithelial stroma. C, D, cGVHD conjunctiva showing attenuated and elongated processes extending into the subconjunctival stroma through disrupted basal lamina (arrows). Fibroblasts in the subconjunctival stroma (D) show an activated form containing large amounts of protein in the rough endoplasmic reticulum (arrowheads). Reprinted with permission from Ogawa et al.[28]

according to the results of prospective validation studies.[37] According to the National Institute of Health Consensus, a diagnosis of cGVHD requires the presence of ≥1 diagnostic clinical sign such as poikiloderma or esophageal web or ≥1 distinctive manifestation such as keratoconjunctivitis sicca confirmed by pertinent biopsy or other relevant tests in the same or another organ. Furthermore, alternative possible diagnoses must be excluded. A clinical scoring system from 0 to 3 is provided for the evaluation of involvement of individual organs and sites. The proposed criteria are easy to understand for all members of the medical transplant team, and it may be pertinent for clinicians to perform prospective validation studies of them.

Baseline profiles of ocular surface and tear dynamics after HSCT in patients with or without cGVHD related to dry eye have been reported. These data are useful for the diagnosis of cGVHD related to dry eye and analyzing the efficacy of topical and systemic treatments for cGVHD related to dry eye (Tables 1 and 2).[38,39]

Early diagnosis is recommended for determining adequate therapy for ocular cGVHD. In this regard, prospective ophthalmic evaluation starting from the pretransplantation period is recommended to diagnose new onset of dry eye or other ocular cGVHD-related and nonGVHD illnesses after HSCT. Therefore, both transplant internist and ophthalmologist should communicate in detail regarding the dose of systemic and local immunosuppressant.[40,41]

Meibomian gland dysfunction (MGD) is a frequent complication after HSCT (Tables 3 and 4).[39,42–44] The presence of MGD may allow physicians to diagnose severe dry eye with GVHD early in the course of the disease. T cell–mediated destruction of meibomian gland epithelia seems to result in MGD. Jester et al[45] showed that hyperkeratinization of the meibomian duct epithelium leading to plugging and dilatation of the duct underlies the development of MGD in rabbits. Keratinization may be another possible mechanism for MGD with GVHD, although the pathogenesis remains controversial. In addition, fibrotic changes of subepithelial stroma may lead

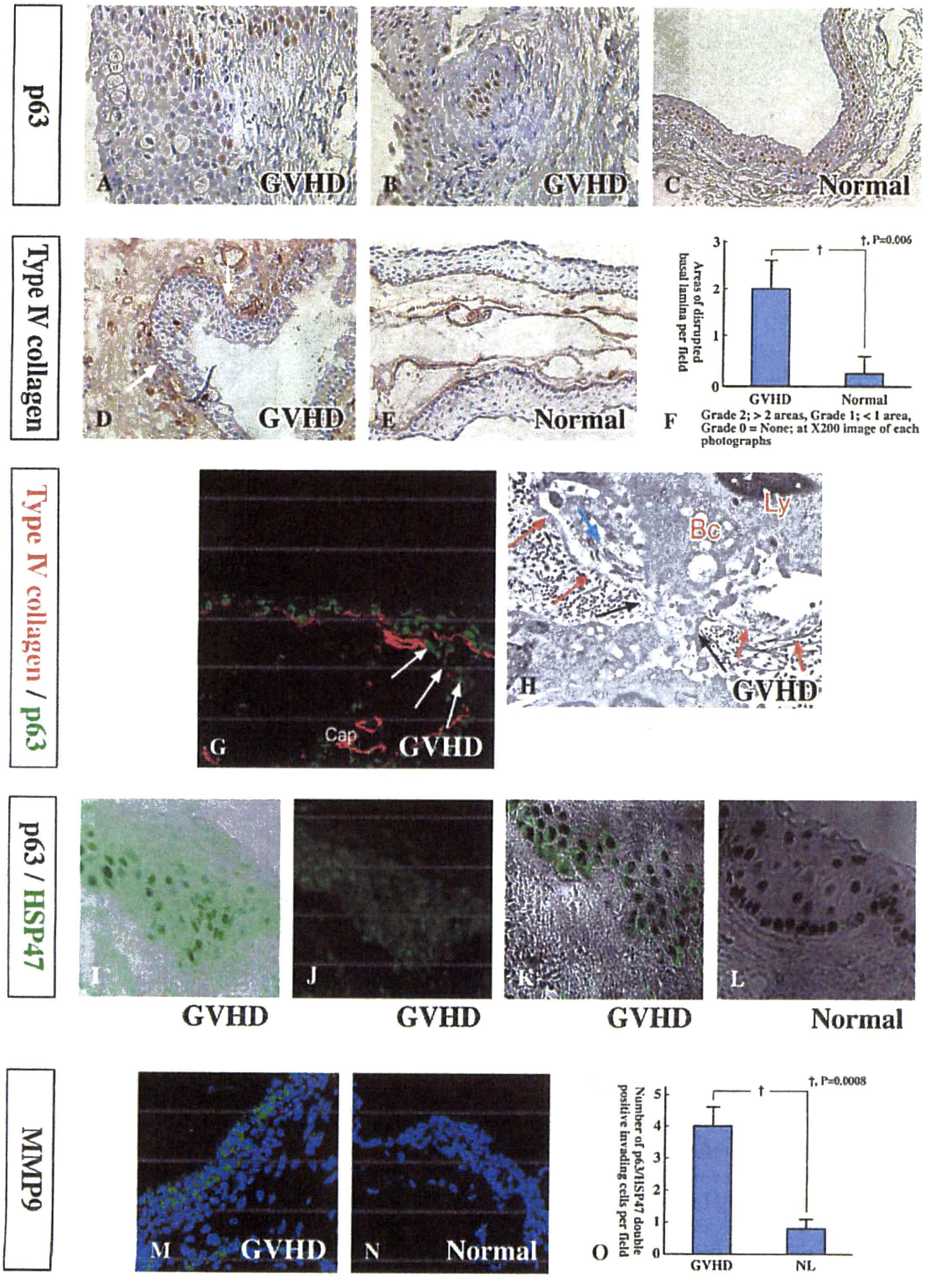

FIGURE 3. Basal epithelial cell invasion through disrupted basal lamina in conjunctival cGVHD. A, B, In cGVHD samples, p63 was found in the nucleus of some cells in the conjunctival stroma. C, In contrast, p63 was found in the nuclei of basal cells in normal conjunctiva. D, Type IV collagen staining revealing disrupted basal lamina in cGVHD conjunctiva (arrows), whereas continuous basal lamina is observable in normal conjunctiva (E). F, The average area of disrupted basal lamina was statistically larger in the GVHD lacrimal gland compared with normal control ($P = 0.006$; Student t test). G, Double staining of p63 (green) and type IV collagen (red). p63$^+$ stromal cells were preferentially located below the type IV collagen layer in regions where this layer showed degradation (arrows). H, Electron micrograph showing cytoplasmic processes of conjunctival basal cells extending into the stroma

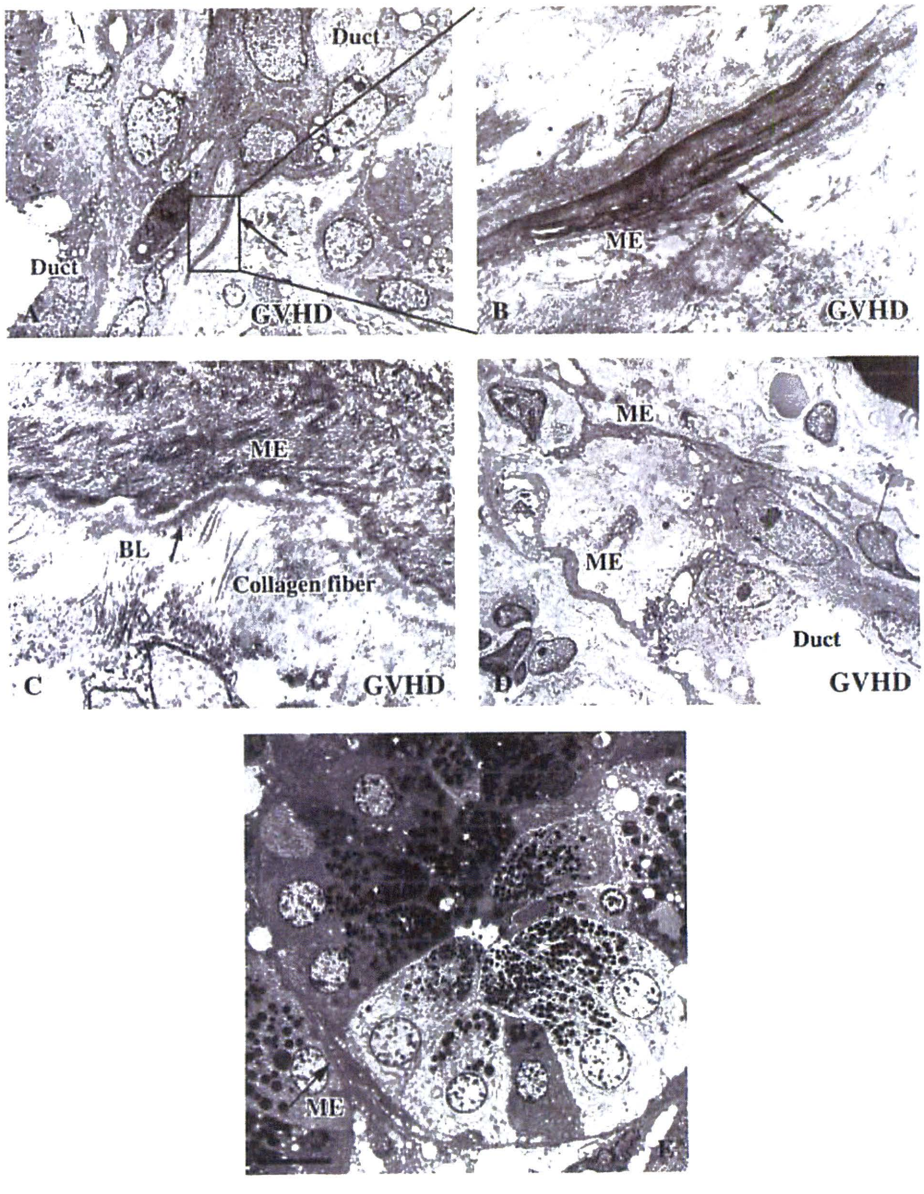

FIGURE 4. Myoepithelial cell invasion in lacrimal gland cGVHD through disrupted basal lamina (BL). Electron microscopy revealed remarkable changes in microfilament cytoskeleton (A, B) and elongation of cytoplasmic processes along its apicobasal axis into subepithelial stroma (arrows) (B). C, Abundant collagen bundles adjacent to myoepithelium (ME). BL, basal lamina (arrow). D, Elongated processes of myoepithelium extending into the subepithelial stroma. E, Normal lacrimal gland showing lymphocytes. ME, myoepithelium (arrow). Original magnification: ×2000 (A, D); ×15,000 (B, C); and ×5000 (E). A–D, Reprinted with permission from Ogawa et al[28]; E, Provided by Dr Hiroto Obata, Jichi Medical University, Tochigi, Japan.

to obstruction of meibomian glands because MGD of cGVHD is often accompanied by conjunctival cicatricial changes.[42,43]

The cornea is a frequently affected organ after HSCT. Dai et al[46] first reported engraftment syndrome–related marginal keratitis in HSCT recipients without GVHD. Cytarabine is often used as conditioning regimen for immune suppression before HSCT, and cytarabine-induced keratoconjunctivitis may occur during or just after the procedure. Clinicians noting keratitis due to any etiology before HSCT should pay careful attention to this complication and apply adequate prophylaxis. Herpes simplex is a commonly occurring viral infection in immunocompromised patients during the early phase after HSCT,[47] which may be avoided by systemic administration of acyclovir in those at risk.

through disrupted basal lamina (black arrows). Blue arrow, collagen bundles in cytoplasm of basal cell. Red arrows, basal lamina. I–K, Double staining revealing basal cells and subconjunctival cells expressing both nuclear p63 (diaminobenzidine staining) and cytoplasmic HSP47 (green) in patients with cGVHD. L, In contrast, double-positive cells were not detected in the basal or stromal cells from normal conjunctiva. M, N, MMP9 expressed in conjunctival epithelia but not in normal conjunctiva. O, The number of p63/HSP47 double-positive invading cells in cGVHD conjunctival samples was significantly higher than that in normal samples (NL) ($P = 0.0008$; Student t test). Original magnification: A, B, G, I–L, ×400; C–E, ×200; and H, ×15,000. Cap, capillary; Bc, basal cell; Ly, lymphocyte. Reprinted with permission from Ogawa et al.[28]

TABLE 1. Scores of Tear Function, Corneal Sensitivity, and Vital Staining in Patients with and without CGVHD-Related Dry Eye after HSCT

	Tear Evaporation Rate ($\times 10^{-7}$ g/cm^2·s)	CS (mm)	Schirmer Test (mm)	TBUT (s)	FS (Points)	RB (Points)
Normal controls	2.2 ± 1.53	60.0 ± 0.0	16.4 ± 11.8	8.9 ± 3.2	0.5 ± 0.7	0.1 ± 0.3
Post-HSCT without dry eye	4.4 ± 2.1	57.5 ± 4.6	14.7 ± 10.3	10.0 ± 0.0	0.3 ± 1.0	0.3 ± 1.0
cGVHD-related mild dry eye	3.6 ± 1.7	57.3 ± 4.2	13.1 ± 11.1	4.9 ± 2.2*†	2.4 ± 1.9*†	1.8 ± 1.9*
cGVHD-related severe dry eye	6.0 ± 3.6*	55.0 ± 7.8*	2.5 ± 2.3*†‡	2.7 ± 1.4*†	5.6 ± 2.6*†‡	5.6 ± 2.1*†‡

*$P < 0.05$ versus normal controls (Kruskal–Wallis test).
†$P < 0.05$ versus post-HSCT without dry eye patients (Kruskal–Wallis test).
‡$P < 0.05$ versus cGVHD-related mild dry eye patients (Kruskal–Wallis test).
TBUT, tear breakup time; FS, fluorescein score; RB, rose bengal score; CS, corneal sensitivity.
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There have been several reports concerning corneal perforation leading to blindness in the setting of cGVHD. As such, stromal melting may be due to immunological reaction involving $CD8^+$ cytotoxic T cells infiltration.[48] Limbal stem cell deficiency and neurotrophic ulcer have been suggested as possible triggers of corneal erosion due to cGVHD.[49]

Vernal conjunctivitis and atopic conjunctivitis have been reported in 0.6% of recipients after HSCT by adoptive transfer of donor T cells and B cells.[50] Anecdotally, we have noted that preexisting symptoms of allergic conjunctivitis sometimes decreased or disappeared after HSCT. This phenomenon may be due to immune reconstitution and replacement by donor immune cells. It is important to evaluate the palpebral conjunctiva and bulbar conjunctiva in detail because dry eye and cicatricial palpebral conjunctivitis are linked to superior limbic keratoconjunctivitis and episcleritis in conjunction with cGVHD.[51,52]

Full-thickness squamous cell carcinoma with dysplasia of conjunctiva was reported as a de novo post-HSCT malignancy.[53] Secondary cancers, most often malignant melanoma, may occur in ≤10% of allogeneic HSCT recipients—especially those with GVHD undergoing conditioning regimens with total body irradiation.[54] Therefore, clinicians should be vigilant and examine in detail pigmentation of the ocular surface.

Mucous membrane pemphigoid can occur after HSCT. So far, 2 cases involving ocular and oral mucosal membrane have been reported in the literature.[55,56] Extensive mucosal blistering in susceptible patients after HSCT is an early warning sign.

Cataracts often develop after HSCT because of long-term use of systemic corticosteroids as preventive strategy against acute graft-versus-host disease and cGVHD. In 1 series, 95 of 248 (38.3%) patients with chronic myelogenous leukemia developed cataract after HSCT.[57] Balaram et al[58] have shown that in allogeneic HSCT recipients, phacoemulsification leads to good visual outcome as long as coexisting ocular surface disease is adequately managed before the surgery. Glaucoma is a known side effect of corticosteroid therapy given to HSCT recipients, with a self-reported incidence rate of 1.6% (occurring in 4 of 248) in a cohort of patients with chronic myelogenous leukemia.[57]

Post-HSCT ptosis has a tendency to occur in female recipients. Univariate analysis revealed that antiacetylcholine receptor antibody titers were higher in patients with cGVHD

TABLE 2. Comparison of Conjunctival Goblet Cell Density (GCD) and Epithelium Squamous Metaplasia (Nelson Grade) in Patients with Dry Eye after HSCT

	GCD (Cells/mm^2)	Squamous Metaplasia (Nelson Grade)
Normal controls	1313.1 ± 733.8	0.7 ± 0.5
Post-HSCT without dry eye	1030.0 ± 433.1	0.7 ± 0.5
cGVHD-related mild dry eye	706.5 ± 583.5*	0.7 ± 0.6
cGVHD-related severe dry eye	396.4 ± 381.0*†	1.6 ± 0.7*†‡

*$P < 0.05$ versus normal controls (Kruskal–Wallis test).
†$P < 0.05$ versus post-HCT without dry eye patients (Kruskal–Wallis test).
‡$P < 0.05$ versus cGVHD-related mild dry eye patients (Kruskal–Wallis test).
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TABLE 3. Clinical Characteristics of Patients with and without Dry Eye after Allogeneic HSCT

Parameter	Without Dry Eye (n = 22)	Mild Dry Eye (n = 12)	Severe Dry Eye (n = 10)	P
Median age (yrs)	28	40	32	—
Age range (yrs)	17–38	24–46	21–43	—
Sex, M/F (%)	15/7 (68.2/31.8)	9/3 (75.0/25.0)	4/6 (40.0/60.0)	—
Median onset (d)	—	149 ± 63	196 ± 115	—
Donor source, n (%)				—
Related	17 (68.2)	7 (58.3)	5 (50.0)	—
Unrelated	5 (31.8)	5 (41.7)	5 (50.0)	—
aGVHD (+), n (%)	11 (50.0)	9 (75.0)	8 (80.0)	NS
aGVHD (−), n (%)	11 (50.0)	3 (25.0)	2 (20.0)	—
cGVHD (+), (%)	8 (36.4)	9 (75.0)	10 (100.0)	<0.001
cGVHD (−), n (%)	14 (63.6)	3 (25.0)	0 (0.0)	—
MGD (+), n (%)	5 (22.7)*	6 (50.0)†	10 (10.0)*†	—
MGD (−), n (%)	17 (77.3)	6 (50.0)	0 (0.0)	—
MGD score points (SD)	0.4 (0.7)*	0.7 (0.9)‡	1.8 (0.4)*‡	—

*$P < 0.001$ (ANOVA followed by Scheffer test).
†$P < 0.05$ (ANOVA followed by Scheffer test).
‡$P < 0.005$ (ANOVA followed by Scheffer test).
aGVHD, acute graft-versus-host disease; NS, nonsignificant difference.
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TABLE 4. Correlations between Dry Eye/MGD and cGVHD

	cGVHD (−) (n = 17)	cGVHD (+) (n = 27)	P*
Dry eye (+), n (%)	3 (17.7)	19 (70.4)	<0.005
Dry eye (−), n (%)	14 (82.3)	8 (296)	
Dry eye severity, n (%)			
Severe	0 (0.0)	10 (37.4)	<0.001
Mild	3 (17.7)	9 (33.3)	–
None	14 (82.3)	8 (29.6)	
MGD (+), n (%)	4 (23.5)	17 (63.0)	<0.05
MGD (−), n (%)	13 (76.5)	10 (37.0)	
MGD score points (SD)	0.3 (0.6)	1.1 (0.9)	<0.005

*Wilcoxon rank sum test.
cGVHD, cGVHD in organs other than eyes.
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than in control group individuals. The etiology may be related to neuromuscular transmitter block seen in autoimmune process.[59]

TREATMENT OF OCULAR cGVHD AND OTHER COMPLICATIONS AFTER HSCT

Dry eye is a hallmark of ocular cGVHD that has significant impact on patients' quality of life. Although there are 2 types of dry eye after HSCT[44]—a mild form and a severe form[17,60]—treatment for mild dry eye associated with cGVHD generally follows that given for common mild dry eye.[60] Strategies for the management of ocular cGVHD include increasing lubrication with artificial tears, topical ointment, controlling evaporation with eyelid management, and draining control with punctal plug or surgical occlusion; ocular surface inflammation may be decreased by immunosuppressants.[11,51,60,61]

Topical corticosteroid therapy for organ-specific GVHD can reduce the need for systemic immune suppression, which may benefit patients in whom GVL is desired.[52] Robinson et al[52] noted that fibrotic lesions start to appear along the superior border of the tarsus where accessory glands are located, resulting in aqueous tear deficiency; these researchers usefully proposed a grading system of conjunctival fibrosis, which is relatively difficult to notice in daily ophthalmic examination. It is important that ophthalmologists be aware that long-term use of corticosteroids may cause glaucoma and cataract as unwanted side effects and that there is no benefit to use topical corticosteroid for cytarabine-induced keratoconjunctivitis.[62]

So as to avoid side effects associated with long-term use of topical corticosteroids, switching to topical immune suppressive agent is recommended. Topical cyclosporine is thought to be partially effective for cGVHD-related dry eye.[38,63,64] Topical tacrolimus ointment may also be useful against dry eye in patients who are refractory to conventional therapy.[65] Surgical undermining of episcleral cyclosporine implant is a novel sustained-release treatment for local drug delivery at therapeutically effective levels that has shown promise preclinically in animal models of cGVHD-related lacrimal gland involvement.[66]

Autologous serum eyedrop and umbilical cord serum eyedrop have been shown effective for improving severe dry eye associated with cGVHD, with no major side effects reported.[67–69] However, it is recommended to evaluate contamination of autologous serum eyedrop if long-term use is planned.[70] Pilocarpine stimulation may be effective for ocular surface in some cases.[71]

There is no specific therapy for ocular fibrosis at present. In this regard, topical tranilast may be useful for the treatment of early dry eye associated with cGVHD through inhibiting TGF-β signaling for ocular fibrosis, although further study is needed to confirm the efficacy.[72] In severe cases, amniotic membrane transplantation may be useful. This reduces ocular fibrosis and inflammation through blockade of TGF-β signaling and inflammation through interleukin 10 secreted by amniotic membrane.[73] Autologous serum-derived cultivated corneal epithelial transplantation for severe ocular GVHD with limbal stem cell deficiency has provided good prognosis.[74]

For the management of severe dry eye secondary to cGVHD, medical use of contact lenses such as silicone hydrogel contact lenses and sclera lenses has been effective in the relatively short-term.[75,76] Intensive care of therapeutic contact lens use is required for prevention of opportunistic infections related to immunosuppressive treatment.

Recent advances of HSCT and combined therapy with biological agents such as imatinib through dual inhibition of TGF-β and platelet-derived growth factor signaling pathway for fibrosis[77] and rituximab targeting B cells[78] may contribute to better outcome of late complications after HSCT. Although several supportive therapies can reduce ocular symptoms, specific therapies that suppress immune processes and pathogenic fibrosis in ocular lesions are necessary to control dry eye related to cGVHD. In addition, detailed evaluation of new therapies for ocular cGVHD should be done by prospective, randomized, case-controlled studies.

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ORIGINAL ARTICLE

Baseline profiles of ocular surface and tear dynamics after allogeneic hematopoietic stem cell transplantation in patients with or without chronic GVHD-related dry eye

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We evaluated ocular surface alterations in allogeneic hematopoietic stem cell transplantation (HSCT) recipients with or without chronic GVHD-related dry eye in a prospective study. Fifty eyes of 25 post-HSCT patients and 28 eyes of 14 age-matched healthy controls were included. Meibomian gland (MG) obstruction, tear evaporation rate, corneal sensitivity (CS), Schirmer test-I, tear break-up time (BUT) and ocular surface vital staining were examined. Conjunctival impression and brush cytology specimens were collected to evaluate the goblet cell density (GCD) and the inflammatory cell numbers. Obvious MG obstruction, decreased CS and enhanced tear evaporation rate were found in post-HSCT patients compared with normal controls. In addition, decreased conjunctival GCD, increased conjunctival squamous metaplasia and inflammatory cells were noted in cGVHD-related dry eyes compared with normal controls and post-HSCT without dry eye subjects. Furthermore, the conjunctival inflammatory cells were significantly higher in severe dry eyes compared with mild dry eyes ($P = 0.03$). We found comprehensive ocular surface alteration in post-HSCT patients, regardless of whether they had cGVHD-related dry eye or not. The results suggest that the extent of inflammatory process seems to have a pivotal role in the outcome of the cGVHD-related dry eye.
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Introduction

Chronic GVHD is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT).[1] Ocular surface is one of the target tissues of cGVHD. About 50% of patients develop dry eye or experience a worsening of the pre-existing dry eye after HSCT.[2] Dry eye is a distinctive sign and symptom for the diagnosis of cGVHD.[1] However, the pathogenesis of dry eye associated with cGVHD is still unclear, and effective treatments have not yet been established.[3] Pathogenic studies of dry eye associated with cGVHD depend on the lacrimal gland and conjunctival biopsy.[4–6] It is impossible to follow the alterations of the ocular surface pathologic process after HSCT by repeated biopsy. On the other hand, impression cytology and brush cytology are widely used methods to evaluate the ocular surface pathologic changes.[7] They are noninvasive, repeatable, and useful in following the changes in the ocular surface.[8,9] However, there are few reports on impression cytology changes and brush cytology characteristics in patients with cGVHD-related dry eye.[10] On the other hand, the conditioning regimen including total body irradiation and high incidence of meibomian gland dysfunction (MGD) in post-GVHD patients contributes to the ocular surface and tear function changes.

However, there is no report comparing the tear functions and ocular surface alterations between post-HSCT patients with or without dry eye. In a previous study,[2] we noticed there were two types of dry eye after HSCT. One had severe ocular surface and tear function damage with decreased reflex tearing that occurred soon after the onset of dry eye, whereas the other was mild with normal reflex tearing. There are no data comparing the ocular surface and tear function differences between these two types of dry eye.

Patients and methods

Patients

Fifty eyes of 25 patients who underwent HSCT were enrolled at the dry eye clinic at Keio University from January 2006 to December 2006. Included were 20 eyes of 10 patients (5 males and 5 females; range, 30–66; median, 50 years) with cGVHD-related severe dry eye, 20 eyes of 10 patients (6 males and 4 females; range, 37–62; median, 51 years) with cGVHD-related mild dry eye and 10 eyes of 5 patients (3 males and 2 females; range, 39–50; median, 45 years) without dry eye. All the patients had no previous conjunctival or corneal disease or infections or other ocular disease at clinical examination. Twenty-eight eyes of 14 healthy subjects (10 males and 4 females; range, 20–70; median, 39 years) were also recruited as normal controls. The control subjects did not have any history of ocular or systemic disease or a history of topical eye drops or contact lens use that would alter the ocular surface as well. According to the global diagnostic criteria of dry eye, and the severity grading of the Dry Eye Workshop Report 2007,[11,12] we diagnosed the patients as having dry eye when patients had any sign of tear film instability (tear break-up time (BUT) $\leqslant 5$ s, Schirmer test $\leqslant 5$ mm), any abnormality of the ocular surface (Rose Bengal score $\geqslant 3$, Fluorescein score $\geqslant 1$) and/or symptoms of ocular irritation. Severe dry eye was defined as previously described[2,13] In brief, patients were diagnosed as having severe dry eye if the Schirmer test with nasal stimulation (reflex tearing) was $\leqslant 10$ mm, and the FS and RB scores were $\geqslant 3$ points and/or grade 3 and 4 according to the DEWS report 2007. The study was carried out in accordance with the principles of the Declaration of Helsinki. Informed consents and ethics board reviews for the examination procedure were obtained.

Clinical examinations

The ocular surface was examined by the double vital staining method. Two microliters of a preservative-free combination of 1% Rose Bengal and 1% fluorescein was instilled in the conjunctival sac by a micropipette.[14] The staining of Rose Bengal was scored for the temporal and nasal conjunctiva and the cornea, on a scale of 0–3 points. Fluorescein staining score also ranged between 0 and 9 points, but only for the cornea.[15] The BUT value was measured three times at the time of double staining, and the mean value was used for calculation. Schirmer I test was performed with standardized strips of filter paper (Alcon Inc., Fort Worth, TX, USA). To evaluate the obstruction of the MG orifice, digital pressure was applied on the tarsus. The expression of meibomian secretion (meibum) was scored as follows:[16] grade 0, clear meibum is easily expressed; grade 1, cloudy meibum is expressed with mild pressure; grade 2, cloudy meibum is expressed with more than moderate pressure; and grade 3, meibum cannot be expressed even with hard pressure.

Tear evaporimetry

The tear evaporation was measured with the evaporimeter (KAO Corporation, Tokyo, Japan).[17] Briefly, the eyecup of the evaporimeter tightly covered the subject's eye, and then the device measured the tear evaporation rate in both eyes-closed and eyes-open conditions. In this way, we can eliminate the evaporation from the eyelid. The computer system calculated the difference between these two conditions and gave the tear evaporation rate. The unit of tear evaporation rate is 10^{-7} g/cm^2 s.

Corneal sensitivity

Measurement of corneal sensitivity (CS) was performed using a Cochet-Bonnet aesthesiometer. The measurements were begun with the nylon filament fully extended. The tip of the nylon filament was applied perpendicularly to the surface of the cornea making certain not to touch the eyelashes and was pushed until the fiber's first visible bending. The length of the fiber was gradually decreased until a blink reflex was observed. The length was recorded in units of millimeters. Measurements were taken from the central cornea and the mean of the measurements was recorded as the CS reading of that eye.[18,19]

Conjunctival impression cytology

The impression cytology samples were obtained under topical anesthesia with 0.4% oxybuprocaine. A piece of cellulose acetate filter paper (Millipore HAWP 304, Bedford, MA, USA) was put on the temporal bulbar conjunctiva and gently pressed by forceps for several seconds. The specimens were fixed with 10% formalin neutral buffer solution and stained with Periodic Acid-Schiff (PAS). Five nonoverlapping areas of $\times 400$ magnification were randomly selected and photographed. The goblet cell density (GCD) was reported as cells per square millimeter. The conjunctival epithelial squamous metaplasia was evaluated according to Nelson's grading scheme.[20]

Conjunctival brush cytology

The brush cytology samples were collected after administration of topical anesthesia with 0.4% oxybuprocaine. The central upper palpebral conjunctiva was gently brushed seven times with a disposable dental brush 1.5 mm in diameter (Dentalpro, Jacks, Co., Osaka, Japan). After sampling, the brush was immediately put in 1 ml of Hank's solution and shaken several times to detach the cells from the brush. The suspended cells were centrifuged with cytocentrifuge at 700 r.p.m. for 10 min to make the monolayer cell smears. The slides were stained by diff-quick staining. We counted up to 500 cells including inflammatory cells and epithelial cells in nonoverlapping fields under microscopic observation (magnification, $\times 400$). The inflammation was reported as the number of inflammatory cells in the total number of 500 brush cells.[10]

Statistical analysis

The data were analyzed by Instat (GraphPad Software, San Diego, CA, USA). Mann–Whitney U-test was used to compare the onset duration of dry eye. Kruskal–Wallis H-test was used for the comparisons of clinical examination parameters, tear evaporation rates, GCD, conjunctival squamous metaplasia, and inflammatory cell amount. The probability level of 5% was chosen as the statistical significance.

Results

Demographic characteristics

Patients' demographic characteristics were summarized in Table 1. The onset of dry eye in cGVHD-related severe and mild dry eye was 6.8 ± 2.5 and 13.2 ± 9.1 months, respectively, after HSCT. The onset of dry eye in the severe dry eye group was significantly earlier than the onset in the mild dry eye group ($P = 0.02$). Nine out of 10 severe dry eye patients had systemic cGVHD, but only 3 in 10 mild dry eye patients had systemic cGVHD.

Clinical examination parameters

The baseline scores of CS, ocular surface vital staining and tear function were summarized in Table 2. Obviously decreased CS was found in post-HSCT patients either with or without dry eye, but statistically significant decrease was found only in the severe dry eye group. Although the mean CS in the severe dry eye group was considerably lower than those with mild dry eye and post-HSCT without the dry eye groups, there was no statistically significant difference among the three groups. Obvious MG orifice obstruction (grade >1) was noted in 40 of 50 eyes of the post-HSCT patients as shown in Table 3. MG orifice obstruction degree in post-HSCT patients was statistically higher than normal controls, but there was no significant difference between the three post-HSCT groups. The tear evaporation rate in normal control, post-HSCT without dry eye, mild dry eye, and severe dry eye group was $2.2 \pm 1.53 \times 10^{-7}$ g/cm^{2}s, $4.42 \pm 2.13 \times 10^{-7}$, $3.6 \pm 1.66 \times 10^{-7}$,

Table 1 Demographic characteristics

Case no	*Age (years)*	*Gender*	*Diagnosis*	*Systemic cGVHD*	*Dry eye*	*Onset (month from HSCT to dry eye)*	*Month since HSCT*
1	52	M	MDS	Lung, skin, mouth	Severe	7	29
2	63	M	MDS	Liver, skin, mouth	Severe	6	65
3	64	M	MM	Skin, mouth	Severe	7	30
4	35	F	CML	Mouth	Severe	7	61
5	30	F	AML	Mouth	Severe	7	93
6	50	M	AML	Mouth, skin, liver	Severe	3	69
7	36	M	CML	Mouth	Severe	7	88
8	66	F	MM	Mouth, skin	Severe	11	58
9	57	F	MDS	Mouth, skin	Severe	10	34
10	34	F	ALL	( )	Severe	3	144
11	49	M	ALL	Lung, liver, skin	Mild	9	24
12	54	M	ALL	Mouth, skin, intestinal	Mild	12	66
13	59	F	ALL	(−)	Mild	11	96
14	56	M	MDS	Liver	Mild	36	156
15	62	F	ALL	(−)	Mild	2.5	19
16	37	M	MDS	(−)	Mild	5	28
17	45	M	MDS	(−)	Mild	12	12
18	61	F	ALL	(−)	Mild	16	36
19	51	M	AML	(−)	Mild	15	36
20	58	F	NHL	(−)	Mild	13	30
21	45	M	AA	(−)	(−)	(−)	60
22	44	F	AML	(−)	(−)	(−)	120
23	39	F	CML	(−)	(−)	(−)	144
24	50	M	AML	(−)	(−)	(−)	30
25	48	M	AML	(−)	(−)	(−)	3

Abbreviations: AA = aplastic anemia; cGVHD = chronic GVHD; F = female; HSCT = hematopoietic stem cell transplantation; M = male; MDS = myelodysplastic syndrome; MM = multiple myeloma; NHL = non-Hodgkin lymphoma.

Table 2 The scores of tear functions, corneal sensitivity and vital stainings

	Tear evaporation rate ($\times 10^{-7}$g/cm^{2}s)	*CS (mm)*	*Schirmer test (mm)*	*BUT (s)*	*FS (points)*	*RB (points)*
Normal controls	2.2 ± 1.53	60	16.35 ± 11.82	8.92 ± 3.17	0.54 ± 0.66	0.13 ± 0.34
Post-HSCT without dry eye	4.42 ± 2.13	57.5 ± 4.63	14.7 ± 10.34	10	0.3 ± 0.95	0.3 ± 0.95
cGVHD-related mild dry eye	3.6 ± 1.66	57.25 ± 4.16	13.06 ± 11.05	4.85 ± 2.18^{a,b}	2.4 ± 1.93^{a,b}	1.8 ± 1.85^{a}
cGVHD-related severe dry eye	5.98 ± 3.61^{a}	54.98 ± 7.75^{a}	2.45 ± 2.28^{a,b,c}	2.68 ± 1.4^{a,b}	5.6 ± 2.56^{a,b,c}	5.55 ± 2.06^{a,b,c}

Abbreviations: BUT = tear break-up time; cGVHD = chronic GVHD; FS = fluorescein score; HSCT = hematopoietic stem cell transplantation; RB = Rose Bengal score.

$^{a}P < 0.05$, compared with normal controls, Kruskal–Wallis test.

$^{b}P < 0.05$, compared with post-HSCT without dry eye patients, Kruskal–Wallis test.

$^{c}P < 0.05$, compared with cGVHD-related mild dry eye patients, Kruskal–Wallis test.

Table 3 Comparison of orifice obstruction grade of meibomian gland

Orifice obstruction	*Normal controls*	*Post-HSCT without dry eye*	*cGVHD-related mild dry eye*	*cGVHD-related severe dry eye*
Grade 0	26 (92.86%)	2 (20%)	0	2 (10%)
Grade 1	2 (7.14%)	2 (20%)	4 (20%)	0
Grade 2	0	2 (20%)	7 (35%)	5 (25%)
Grade 3	0	4 (40%)	9 (45%)	13 (65%)

Abbreviations: cGVHD = chronic GVHD; HSCT = hematopoietic stem cell transplantation.

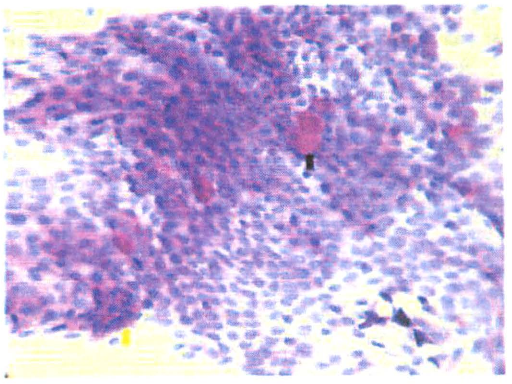

Figure 1 Representative conjunctival impression cytology specimens from a 50-year-old male, post-HSCT without dry eye subject. Note plenty goblet cells (black arrow) and mucin pick up (yellow arrows). Periodic acid-Schiff (PAS) staining, magnification, ×400.

and $5.98 \pm 3.61 \times 10^{-7}$ g/cm^{2}s, respectively. Although the mean tear evaporation rate in mild dry eye and post-HSCT without dry eye patients was higher than in normal controls, statistically increased tear evaporation was found only in cGVHD-related severe dry eye patients ($P < 0.001$).

Conjunctival impression cytology
Conjunctival specimens from normal controls and post-HSCT without dry eye subjects showed plenty of goblet cells and mucin pick up (Figure 1). The goblet cell densities in these two groups were 1313.13 ± 733.82 and 1030 ± 433.14 cells/mm^2. The mean GCD in the cGVHD-related mild and severe dry eye groups was 706.49 ± 583.52 and 396.36 ± 381.00 cells/mm^2. Both were obviously lower than the former two groups without dry eye (Table 4). Moreover, significant conjunctival epithelial squamous metaplasia was noted in severe dry eye patients. The mean grades of squamous metaplasia in normal control, post-HSCT without dry eye, and mild dry eye groups were 0.70 ± 0.46, 0.71 ± 0.52, and 0.72 ± 0.56, respectively. There was no statistical difference among the three groups. However, the average grade of squamous metaplasia in severe dry eye subjects was 1.61 ± 0.72, which was significantly higher than that in the other three groups (Table 4). Except decreased GCD, the PAS staining also showed inflammation in some impression cytology specimens from the cGVHD-related severe dry eye and mild dry eye patients (Figures 2 and 3). In addition, the PAS staining also indicated the intense inflammatory cell infiltration that frequently appeared with the abnormal mucin conglomeration.

Brush cytology
There was no inflammatory cell in the brush cytology specimens from normal controls. In contrast, a different extent of inflammatory cell infiltration was found in the specimens from post-HSCT patients (Figure 4). The mean number of inflammatory cells in 500 brush cells in post-HSCT without dry eye, mild dry eye, and severe dry eye specimens were 5.44 ± 6.04 cells, 14.64 ± 9.75 cells, and 22.64 ± 11.69 cells, respectively. The mean inflammatory cell numbers in both cGVHD-related mild and severe dry eye specimens were significantly higher than in normal controls and post-HSCT without the dry eye group ($P < 0.001$). Moreover, the inflammatory cell number in the severe dry eye group was statistically higher than in the mild dry eye group ($P = 0.03$).

Discussion

In this study, we evaluated the detailed baseline profiles of ocular surface and tear function alterations in post-HSCT patients with or without dry eye disease. We found obviously decreased CS in post-HSCT subjects either with or without dry eye disease. Although the reduction of CS in severe dry eye patients seemed to be more prominent, there were no statistical differences compared with post-HSCT without dry eye and mild dry eye patients. A reduction of CS has been reported in dry eye patients.[18,19] We also noted decreased CS in cGVHD-related dry eye patients in our previous study.[3] Considering the conditioning regimens before HSCT, such as total body irradiation, which includes orbital irradiation, we thought decreased CS in cGVHD-related dry eye patients may not be because of the dry eye pathologic process. Therefore, we recruited post-HSCT without dry eye subjects in this study. According to the present results, decreased CS was obvious even in post-HSCT without dry eye patients. Our study suggested that the conditioning regimens before HSCT may be more responsible for the decreased CS in cGVHD-related dry eye disease.

Moreover, increased MG obstruction grade was found in post-HSCT both with and without dry eye patients. Consistent with this, an increased tendency in the tear evaporation rate was noted in post-HSCT patients. However, the statistical increase was found only in the severe dry eye patients. MGs produce lipid material that spread and cover the ocular surface during the blink to keep the tear film stable and to reduce the tear evaporation. The dysfunction of the MG can induce evaporative dry eye.[21] On the other hand, decreased tear production