Purpose: To evaluate therapeutic effect of multilayer amniotic membrane transplantation (AMT) in conjunction with antiviral and corticosteroid therapy on herpes necrotizing stromal keratitis.

Design: Retrospective interventional case series.

Participants: Fifteen patients (15 eyes) with herpes necrotizing stromal keratitis, persistent corneal inflammation, and impending ulcer, despite topical and systemic antiviral treatment for over 2 weeks.

Methods: Multilayer AMT was performed in the 15 eyes. Antiviral medications and appropriate corticosteroids were administered after surgery. Remodeling of amniotic membrane (AM) and growth of epithelial cells were detected by confocal microscopy.

Main Outcome Measures: Visual acuity and corneal status (ulceration, edema, and opacification).

Results: Follow-up ranged from 7 to 13 months (mean ± standard deviation, 8.9 ± 1.8). Visual acuity improved by ≥2 lines in 14 eyes. Central corneal ulcers healed completely at 2.0 ± 0.6 weeks, and paracentral ulcers at 2.1 ± 0.6 weeks (t = 0.314, P = 0.759). Corneal stromal thickness was restored in eyes with central ulcers at 2.4 ± 1.2 weeks and in those with paracentral ulcers at 2.6 ± 0.7 weeks (t = 0.425, P = 0.678). Superficial epithelial cells, together with small basal epithelial cells, gradually migrated to the surface of AM on postoperative weeks 1 to 3. There were corneal nebulae in 11 eyes, corneal maculae in 3 eyes, and a corneal leukoma in 1 eye at the end of follow-up. No recrudescence occurred in any eye.

Conclusion: Multilayer AMT combined with antiviral and corticosteroid therapy appears effective in treating herpes necrotizing stromal keratitis. It provides patients with marked scars and visual impairment an opportunity for subsequent keratoplasty by arresting the inflammatory response and reducing the graft bed diameter.
fined, it is known that basement membrane may help to epithelialize by promoting adhesion, proliferation, and differentiation, and the stromal matrix is effective in suppressing inflammation and scarring.11 For corneal ulcers caused by neurotrophic matter or others, the actions mentioned above are very desirable. Indeed, single-layer and multilayer AMTs have been used successfully to treat these ulcers.8–15

We report a series of patients with herpes necrotizing stromal keratitis who were treated successfully with AMT combined with antiviral and corticosteroid therapy.

Patients and Methods

Fifteen patients (15 eyes) with herpes necrotizing stromal keratitis were referred to our institution from April 2003 to March 2005. Nine were male and 6 female, with an age range of 30 to 59 years (mean ± standard deviation, 47.0±6.9). All patients visited us within 2 weeks after onset of keratitis. Diagnostic criteria were based on previous reports.16,17 Briefly, the majority of patients had a history of recurrent episodes of corneal disease. Ocular manifestation was necrotizing corneal ulceration with dense infiltration of edematous stroma. These patients had been predisposed to antiviral medications at our institution. Smears and cultures of corneal ulcers in depth without descemetocele, which persisted in 15 eyes were referred to our institution from April 2003 to March 2005. Fifteen patients (15 eyes) with herpes necrotizing stromal keratitis who were treated successfully with AMT were included in this study. The patients were followed up for 7 to 13 months (mean ± standard deviation, 47.0±6.9). All patients visited us within 2 weeks after onset of keratitis. Diagnostic criteria were based on previous reports.16,17 Briefly, the majority of patients had a history of recurrent episodes of corneal disease. Ocular manifestations were stained with an immunoperoxidase assay, using a polyclonal antibody to HSV-1, to detect HSV-1 antigen. We slightly modified the previously reported methods.18,19 Biopore membrane (Millipore Corp., Bedford, MA) was pressed gently on the ulcerated corneal surface for 5 seconds, with contralateral healthy eyes as controls, and harvest cells were fixed by air drying. The specimens, mounted on plastic tube, were incubated with prediluted (1:70) primary antibody (rabbit anti-HSV type I, Dako, Glostrup, Denmark) and secondary antibody conjugated with horseradish peroxidase at 37° C for 20 minutes. Then 50 μl of diaminobenzidine substrate was added and incubated for 2 minutes at room temperature. Light microscopy was used to examine the staining results.

Written informed consent was obtained from each patient. Amniotic membrane was prepared and preserved as previously reported, with minor modifications.20 All surgeries were performed by the same surgeon. Topical anesthesia was given before cellular debris and necrotic corneal tissue were removed from the base and wall of the corneal ulcer. Human AM was placed with basement membrane side up, layer by layer, to fill up the ulcer and cover the defect, and cut to a graft larger than the corneal ulcer by trimming off excess parts. After the graft was secured to the edge of ulceration with interrupted 10-0 nylon sutures, the knots were cut short but not buried into corneal stroma to avoid AM detachment at the time of suture removal. Finally, another superficial AM patch was sutured onto the surface to cover the entire cornea with a continuous 10-0 nylon suture placed within 1 mm of the limbus and 8 to 10 interrupted sutures at superficial sclera. No bandage contact lens was used.

Postoperatively, all eyes were given 1% tobramycin and dexamethasone eyedrops (Alcon, Puurs, Belgium) every 2 hours, pranoprofen eyedrops (Senju, Osaka, Japan) 4 times daily, and 1% tobramycin and dexamethasone ointment (Alcon) at night. Topical 0.1% acyclovir (Shandong Lukan Cisen Pharmaceutical Co., Ltd., Jining, China) was administered 4 times daily for 2 weeks and 2 to 3 times daily thereafter until 2 months after surgery. Corticosteroid drops were reduced to 4 times daily after 1 week and tapered off within 1 month. Corticosteroid ointment was terminated at approximately half a month. Systemic acyclovir was administered 5 times daily for 7 days including preoperative application (40 mg/kg) and for the following 1 to 3 months (20 mg/kg). The specific duration and frequency of administration depended on the individual sensitivity and prognosis of corneal disease.

All patients were examined weekly in the first preoperative month and monthly thereafter. Postoperative examination included visual acuity (VA), intraocular pressure, corneal status (ulceration, edema, and opacification), and AM transformation. Fluorescein staining was used for detection of corneal epithelial defects, and confocal microscopy was performed to determine the AM remodeling and growth of epithelial cells.23 Relying on extents of corneal opacification, we divided it into corneal nebula (iris details visible), corneal macula (iris details partially obscured), and corneal leukoma (iris details obscured).

Student’s t test was used to compare healing time of corneal ulcers and restoration of stromal thickness, respectively. P<0.05 was considered statistically significant.

Results

Immunopathological staining produced positive results in 6 eyes, whereas no viral antigen was found in the specimens of the fellow eyes. The patients were followed up for 7 to 13 months (mean ± standard deviation, 8.9±1.8). At the last visit, VA improved ≥2 lines in 14 eyes. Another eye had VA of below 20/400 for the presence of corneal leukoma, which was attributed to the preoperative ulcer located on the central cornea involving the deep stroma. Intraocular pressures were 15.1±1.8, 16.5±1.1, and 15.9±2.3 mmHg on postoperative days 7, 15, and 30, respectively. Suture removal was started at postoperative 7 to 10 days, when the superficial AM patch dissolved, and was completed at 20 days for the remaining inferior AM grafts.

In eyes with superficial corneal ulcers, stromal edema subsided at 10 days, whereas it required more than 20 days for deep corneal ulcers. Referring to negative fluorescein staining, central corneal ulcers healed completely at 2.0±0.6 weeks and paracentral ulcers at 2.1±0.6 weeks (t = 0.314, P = 0.759). It took 2.4±1.2 weeks for central ulcers and 2.6±0.7 weeks for paracentral ulcers to restore corneal stromal thickness (t = 0.425, P = 0.678). Although paracentral ulcers were more severe than central ones, no obvious difference was observed in the healing of corneal ulcers and cessation of stromal edema. Hypopyon in 2 eyes was absorbed completely on postoperative days 10 and 12, respectively. There were corneal nebula in 11 eyes, corneal macula in 3 eyes, and corneal leukoma in 1 eye at the end of follow-up. No recurrence occurred in any eye. The outcomes are shown in Table 1 (available at http://aaojournal.org) and Figures 1 to 3.

Clear structure of AM grafts without inflammatory cell migration was seen readily by confocal microscopy on postoperative day 7. Healthy superficial and basal epithelial cells surrounding a corneal ulcer gradually migrated to the AM surface on postoper-
ative weeks 1 to 3. Confluent layers of flat and polygonal epithelial progenitor cells regenerated and overlaid on the AM surface, whereas AM covered the corneal ulcer, in a sandwich configuration. Subsequently, AM gradually remodeled and dissolved within no more than 2 months.

Patient 10

A 48-year-old man with a history of repeated red eye presented with foreign body sensation and blurred vision in his left eye for over 2 weeks. He had suffered from similar symptoms with spontaneous relief 6 times. At the onset of this attack, from the misuse of topical steroid in the other hospital, dendritic lesion rapidly progressed into stromal ulcer. Upon referral to our institution, impression cytological examination showed positive HSV antigen (Fig 3A). There was a severe paracentral ulcer, 5×9 mm in diameter and extending to two thirds of the inferior stroma in depth, with an irregular surface (Fig 3B). Despite topical and systemic antiviral medications for more than 1 week, the corneal ulcer continued to deteriorate. After multi-layer AMT, the superficial AM patch dissolved on postoperative day 10 (Fig 3C). Corneal ulcer healed at 3 weeks with the AM graft in a good position (Fig 3D). A stable ocular surface

Figure 1. Patient 1. A, Paracentral corneal ulcer. B, Edematous corneal stroma and multilayer amniotic membrane grafts overlaying corneal ulcer. C, Inferior amniotic membrane graft in place and healing of corneal ulcer on postoperative day 15.

Figure 2. Patient 9. A, Superficial corneal ulcer coupled with local infiltration. B, Inferior amniotic membrane graft with negative fluorescein staining on postoperative day 10. C, Formation of corneal scarring on postoperative month 3.
and irregular nebula were observed 3 months later. Visual acuities were counting fingers before surgery and 20/40 on postoperative month 5.

**Patient 11**

A 45-year-old woman presented photophobia and irritation in her right eye for over 20 days due to unknown reasons. It had been misdiagnosed as bacterial keratitis before referral to our institution, and corneal ulcer exacerbated with antibacterial medications. She had experienced the same episode 2 times. On examination, an irregular central ulcer with keratic precipitates and hypopyon was observed. We performed corneal scrapings at the time of admission and excluded bacterial and fungal infections. Multilayer AMT was performed 5 days after admission. Antiviral agents and topical corticosteroid were given. Profound regression of corneal inflammation and rapid closure of epithelial defects were found on postoperative day 15. Hypopyon was absorbed on postoperative day 10. Corneal ulcer healed 2 weeks after the suture removal, when there was gradual dissolution of the superficial AM layers. Visual acuity improved from preoperative 20/400 to 20/50 at the end of follow-up.

**Discussion**

As previously reported, the management of herpes necrotizing stromal keratitis started from making a correct diagnosis by ruling out bacterial and fungal infections and confirming the diagnosis on the basis of the history of recurrent episodes and the clinical presentation. Most important is that these cases must be responsive to antiviral medications under the condition that reliable experimental assays are lacking. Impression cytological examination in combination with immunopathological examination has significant advantages in the diagnosis of superficial rather than stromal herpetic keratitis. In this study, positive HSV antigen was easily detected in cases with a disease history of a dendritic episode. For those suspicious of bac-
terial or superinfected HSV keratitis, appropriate antimicrobial drugs should be considered. In addition, more attention should be paid to debride the necrotizing tissue around and on the corneal ulcer base completely. Otherwise, replicating virus and severe local inflammatory response may lead to damage of the corneal basal membrane and disturb the normal repair process of epithelial cells. Multilayer AMT comprises an inferior graft, which covers the ulcerated sites and overlaps more than one layer to reconstruct corneal thickness, and a superior patch, which overlays the entire cornea. In the current series, an AM graft provided a healthy substrate, contributed to rapid epithelialization, and significantly reduced ocular inflammation. But for patients with a descemetocele, we would not recommend AMT, because amnion would dissolve or shed before healing of corneal perforation and could not produce a continuous basement effect for such corneal ulcers. Consequently, we propose that antiviral agents and corticosteroids are indispensable to the treatment of AMT for herpes necrotizing stromal keratitis.

Topical administration of corticosteroids can suppress the local immune response induced by virion and speed up the cessation of stromal edema. The Herpetic Eye Disease Study has demonstrated that topical corticosteroid was effective in reducing the persistence or progression of stromal inflammation and shortening the duration of herpes simplex stromal keratitis. However, one of the eligibility criteria for herpetic stromal disease is epithelial defect smaller than 1 mm of the involved eye. The fact indicated that all the cases were nonulcerative herpetic stromal keratitis. For ulcerative necrotizing stromal keratitis, by contrast, topical corticosteroids would lead to a high risk of corneal melting or perforation. An AM patch can form a useful barrier by overlying the surface of a corneal ulcer and can exclude ulcer aggravation for the further use of corticosteroids. Based on the appropriate timing and duration of administration in our series, we did not observe corticosteroids compromising wound healing.

Coupled with significant improvement of VA in the majority of cases, corneal ulcers rapidly healed within 1 month, and anatomical integrity was achieved. No necrosis of stromal keratitis recurred, and corneal neovascularization was not frequently observed during the observation period. There was a report that ulcerative herpetic keratitis recurred after AMT, but whether the cases were necrotizing or nonnecrotizing was unavailable. In addition, Meller and Tseng and Azuara-Blanco et al indicated that AM facilitated proliferation and differentiation of epithelial cells, maintained original epithelial phenotypes, and reduced scarring and inflammation in ocular disease. This indicated that AMT was a much better alternative than tectonic or optical corneal transplantation, for the latter faces a high risk of rejection if performed when the eye is severely inflamed. In this study, one eye had a final VA < 20/400 after the formation of leukemia due to deep central ulceration. Conversely, AMT restored a noninflamed ocular surface in the eye, which was crucial in enhancing the success of further optical corneal transplantation if needed in the future.

Presumably, AM has antiviral and antiinflammatory effects on viral disease. Paradowska et al reported that human placenta contained endogenous tumor necrosis factors and interferons, which possibly gave nonspecific antiviral immunity. Amniotic membrane may express cystatin E, a novel human cysteine proteinase inhibitor, to inhibit proteolytic cleavage required for viral replication including HSV-1. Moreover, in a mouse model of HSV-1 stromal keratitis, AMT was effective in promoting corneal wound healing and reducing inflammation, which may be related to the reduced expression and activity of matrix metalloproteinases (MMP-8 and MMP-9) and increased expression of tissue inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-2). The antiviral properties of AM further confirm a promise of AMT for the treatment of herpes necrotizing stromal keratitis.

Although our series lacked a control group, the adjunctive use of antiviral and steroid therapy with AMT should be recommended in the treatment of herpes necrotizing stromal keratitis for its safety and efficacy. It helps to promote epithelialization of corneal defects, alleviate corneal scars, and restore useful VA. Patients with marked scars and visual impairment may get an opportunity for subsequent keratoplasty after such a treatment for the arrestment of inflammatory response and reduction of graft bed diameter.

References

Table 1. Demographic Data and Outcomes

<table>
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<th>Patient</th>
<th>Age (yrs)/Gender</th>
<th>Recurrence (Times)</th>
<th>Location</th>
<th>Diameter (mm²)</th>
<th>Depth in Stroma</th>
<th>Hypopyon (mm)</th>
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<th>BCVA</th>
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<th>RTS (wks)</th>
<th>STH (Days)</th>
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BCVA = best-corrected visual acuity; CF = counting fingers; CU = corneal ulcer; F = female; HM = hand movements; M = male; N = no preoperative hypopyon; RTS = restoration time of stromal thickness; STH = subsided time of hypopyon in anterior chamber.