

# Reactivation of Herpes Virus After Lamellar Keratoplasty

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**Purpose:** To determine if lamellar keratoplasty in rabbits latently infected with herpes simplex virus type 1 (HSV-1) would stimulate graft recipients to shed virus and induce viral-specific corneal lesions.

**Methods:** Rabbits latently infected with HSV-1 received lamellar allografts in one eye from normal uninfected rabbits and the contralateral eyes served as unoperated controls. Normal rabbits received lamellar grafts from rabbits latently infected with HSV-1. For 1 week after surgery, slit-lamp examination and ocular swab sampling were performed daily to assess viral reactivation.

**Results:** The occurrence of positive swab cultures and corneal epithelial lesions after lamellar keratoplasty was significantly higher in operated eyes of latently infected rabbits when compared to the control eyes. Ocular shedding or recurrent lesions were not observed in the normal rabbits receiving corneal grafts from latently infected donors.

**Conclusions:** These results indicated that lamellar keratoplasty induces HSV-1 shedding and recurrent epithelial lesions in the eyes of rabbits latently infected with HSV-1, which received lamellar grafts, but not in the eyes of normal rabbits given lamellar grafts from HSV-1 latently infected rabbits. It seems that the site of viral latency is not the anterior corneal stroma or the epithelium. **Jpn J Ophthalmol 1999;43:257-261** © 1999 Japanese Ophthalmological Society

**Key Words:** Cornea, herpes simplex virus type 1, lamellar keratoplasty, rabbit, reactivation.

## Introduction

Herpes simplex keratitis (HSK) caused by herpes simplex virus type 1 (HSV-1) is the leading cause of infectious blindness in the world. HSV-1 infects the cornea and colonizes the sensory and autonomic ganglia. One result of HSV latent infection is the potential for unlimited recurrences. A stimulus or "trigger" can cause a recurrence in tissues of ectodermal origin.<sup>1-5</sup> Many different stimuli induce reactivation of latent virus including fever, exposure to sunlight or ultraviolet light,<sup>6</sup> hypothermia and hyperthermia,<sup>7,8</sup> psychological stress, menstruation, pharmacological depression of cellular immunity,<sup>9-11</sup> ocu-

lar injuries by foreign bodies, surgical procedures such as radial keratotomy,<sup>12,13</sup> or iontophoresis of epinephrine, 6-hydroxydopamine, or timolol.<sup>14-19</sup> Frequent recurrences can cause stromal scarring that leads to significantly reduced visual acuity.

Corneal transplantation is the only procedure available to correct the blindness caused by recurrent HSK. Unfortunately, corneal transplantation for HSK has a significantly lower success rate than that for nonherpetic conditions. In addition to immunologic corneal allograft rejection, postoperative recurrence of keratitis is another complication. Because nearly 80% of adults in developed countries have been infected by HSV, patients undergoing keratoplasty have a good chance of receiving a graft from a donor latently infected by HSV-1.

We reported previously on the recurrence of HSV-1 infection after penetrating keratoplasty in

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rabbits.<sup>20,21</sup> There are no reports of the effect of lamellar keratoplasty on HSV-1 reactivation; despite the fact that there are more lamellar keratoplasties performed than penetrating keratoplasties to correct herpetic and nonherpetic induced corneal opacification every year.<sup>22–25</sup> We developed an animal model to investigate the effect of lamellar keratoplasty on HSV-1 reactivation.

## Materials and Methods

### *Experimental Design*

New Zealand white rabbits were divided into two groups: (1) rabbits latently infected with HSV-1 received lamellar grafts in one eye (except rabbit 1) from uninfected donor rabbits, and the contralateral eye was used as an unoperated control; (2) normal rabbits received lamellar grafts from rabbits latently infected with HSV-1. Rabbits were inoculated with HSV-1 (McKrae strain) and monitored for spontaneous reactivation. The keratoplasty procedure was done 40 or more days post infection (PI). The corneas were evaluated by slit-lamp examination (SLE) to determine the condition of the graft and occurrence of herpetic keratitis for 7 days after operation. Swabs of eyes were also cultured for detection of infectious virus.

### *Establishment of Latency*

HSV-1 (McKrae strain) was propagated on primary rabbit kidney (PRK) cell monolayers and titrated by plaque assay on monkey kidney cell monolayers. The virus was frozen in small aliquots at  $-70^{\circ}\text{C}$  and the same batch was used for inoculation of all rabbits. Rabbits (1.5–2.5 kg) were bilaterally inoculated with 25  $\mu\text{L}$  of a suspension of HSV-1 ( $3 \times 10^6$  PFU/mL). The corneas were not scarified, but the eyelids were closed and massaged for 30 seconds with care taken to avoid leakage of the viral suspension. Primary corneal infection was verified by SLE using 0.1% fluorescein after 3 days of infection for the appearance of corneal lesions corresponding to herpetic keratitis. Rabbits were followed until all corneas exhibited lesions. They were also examined on PI day 19 to verify the absence of lesions. Rabbits in which the lesions resolved were assumed to be latently infected.<sup>20</sup> The care and maintenance of rabbits used in this study conformed to the ARVO Resolution on the Use of Animals in Research.

### *Collection and Culturing of Tear Film*

Tear film was collected from each rabbit eye using a sterile Dacron-tipped swab starting at PI day 20

and continuing through PI day 39, as described previously by Berman and Hill.<sup>26</sup> Briefly, the swab was placed in both the upper and lower cul-de-sac and gently rotated and allowed to absorb the tear film in the lower fornix for 5 seconds; the swab was also passed over the cornea. Swabs were placed in tissue culture tubes containing confluent PRK cell monolayers in DMEM with 2% FCS and incubated at  $37^{\circ}\text{C}$  in an atmosphere of 5%  $\text{CO}_2$ . Swabs were removed 24 hours after collection. After lamellar keratoplasty, the tear film was collected in a similar fashion with the exception of swabbing over the corneal graft. All tubes were monitored daily for 7–9 days for the appearance of cytopathic effect indicative of HSV-1.

### *Lamellar Keratoplasty*

Rabbits were anesthetized by intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (10 mg/kg). The eye to undergo lamellar keratoplasty was stabilized by placing sutures under the superior and inferior rectus muscles. The lamellar graft was outlined by 7.0 mm trephine, the edge of trephine cut was held, and the corneal graft was lamellarly dissected by a razor blade at a depth of 0.5 mm parallel to the corneal surface. The same technique was used to harvest both the donor and the recipient corneal lamellas. The transplant was sutured with four 10-0 nylon cardinal sutures at 3, 6, 9, and 12 o'clock, and a 16-bite running suture. The cardinal sutures were removed at the end of the procedure. The running suture was removed 14 days after lamellar keratoplasty.

## Results

The background level of spontaneous viral shedding was determined by collecting tear film on days 20–39 after infection. Eight rabbits with infectious virus found in both eyes were used in this study, seven normal rabbits were transplanted by corneal grafts from HSV-1 latently infected rabbits.

All transplanted rabbits were bilaterally examined daily for 7 consecutive days for the presence of herpetic lesions. In the latently infected rabbit group, the ratio of epithelial lesion days observed to the total observations was 6:63 (9.5%). The lesions observed were either dendritic or geographic epithelial defects (Table 1). Superficial punctate keratitis was not recorded in this study. The ratio of positive tear film cultures to total cultures of tear film was 27:62 (43.5%). All six lesions were associated with positive tear film cultures. The eyes in latently infected rab-

**Table 1.** Herpetic Epithelial Lesions and HSV-1 Ocular Shedding After Lamellar Keratoplasty; Latently Infected Rabbits Received Lamellar Allografts From Uninfected Normal Rabbits (Epithelial Lesions/Tear Film Cultures)

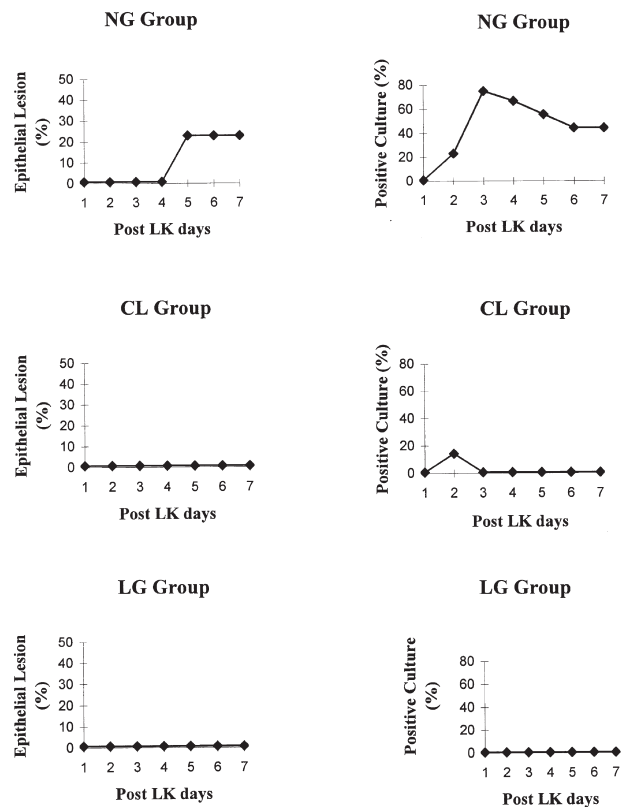
Rabbit Number	Postinfection Day When LK Performed	Eye	Days Post Operation						
			1	2	3	4	5	6	7
1	82	OD	N/-	N/-	N/-	N/-	N/-	N/-	N/-
	82	OS	N/-	N/+	N/*	N/+	N/+	N/+	N/+
2	51	OD	N/-	N/-	N/+	N/+	D/+	D/+	D/+
3	85	OD	N/-	N/-	N/+	N/+	N/+	N/+	N/+
4	89	OS	N/-	N/-	N/-	N/-	N/-	N/-	N/-
5	98	OS	N/-	N/-	N/+	N/+	G/+	G/+	G/+
6	53	OD	N/-	N/-	N/+	N/+	N/-	N/-	N/-
7	53	OD	N/-	N/-	N/+	N/-	N/-	N/-	N/-
8	92	OS	N/-	N/+	N/+	N/+	N/+	N/-	N/-

Eight rabbits underwent lamellar keratoplasty; eyes were swabbed after operation for seven consecutive days for tear film cultures. Only rabbit number 1 received bilateral LK, others received unilateral keratoplasty. N: no lesion; D: dendritic lesion; G: geographic lesion; -: HSV-1 negative culture swab; +: HSV-1 positive culture swab; \*: culture contaminated.

bits that did not undergo keratoplasty did not develop herpetic lesions during the test period. The ratio of positive cultures to total cultures was 1:48 (2.1%). Normal rabbits that received grafts from latently infected rabbits did not develop epithelial lesions or shed virus (data not shown). The difference in the number of epithelial lesion days observed between the latently infected eyes that received normal lamellar grafts and contralateral unoperated controls was statistically significant ( $P < .05$ ). The difference between latently infected rabbits that received normal lamellar grafts and normal rabbits that received grafts from latently infected rabbits ( $P < .05$ ,  $\chi^2$  test) was also statistically significant. The differences in occurrence of positive cultures between latently infected eyes that received normal lamellar grafts and the other two groups were also significant ( $P < .0001$ ,  $\chi^2$  test) (Figure 1).

### Discussion

Lamellar keratoplasty (LK) has been used to treat corneal cap problems related to keratomileusis, keratoconus, granular corneal dystrophy, inflammatory corneal ulceration, and even corneal perforation.<sup>23,24</sup> LK or deep lamellar keratoplasty is thought to be an effective treatment for eyes in which endothelial cell function has been preserved. The rapid postoperative recovery and the low morbidity associated with LK make it preferable to penetrating keratoplasty. Many ophthalmologists recommend LK as the primary procedure to be used to rehabilitate a cornea in which pathology is limited to the an-



**Figure 1.** In NG group, HSV-1 latently infected rabbits received lamellar grafts from normal rabbits. In CL group, contralateral eyes of rabbits in NG group were used as unoperated controls. In LG group, normal rabbits received lamellar grafts from HSV-1 latently infected rabbits. Differences in occurrence of positive cultures between latently infected eyes that received normal lamellar grafts and the other two groups were significant ( $P < .0001$ ,  $\chi^2$  test).

terior portion of this tissue. LK is particularly applicable in younger patients in whom multiple procedures may be necessary over a lifetime because of the possible recurrence of the pathology.<sup>22,25</sup> We have developed a rabbit model of LK to determine the effects of this procedure on HSV-1 reactivation. Rabbits shown to be latent by positive swabs after inoculation underwent LK. The transplants were carried out using normal corneal tissues into latent rabbits and possible latent corneal tissues into normal rabbits. Control corneas were assessed as well.

In our observations, the LK procedure on latently infected rabbits induced HSV-1 ocular shedding and recurrent epithelial lesions. This could be explained by surgical trauma. Stress associated with surgery (including anesthesia stress) may also stimulate HSV-1 reactivation and could be responsible for inducing the rabbits to shed virus into the tear film.<sup>12,13,27</sup> In this study, surgical trauma was limited to the operated eye. Although both eyes were in the same stress environment, the unoperated eyes of latent rabbits did not shed virus after surgery. Many cornea surgical procedures are proven to induce herpetic recurrences. In previous publications, we have reported that corneal incisions for radial keratotomy and penetrating keratoplasty in latently infected rabbits significantly increased the rate of HSV-1 ocular shedding and HSV-1-specific corneal epithelial lesions.<sup>12,20</sup>

Our results also demonstrated that LK did not induce epithelial lesions or viral shedding in normal rabbits that received grafts from latently infected rabbits. This may be due to the possibility that the site of viral latency is not the anterior corneal stroma or epithelium. Therefore, there is no virus present in the transplanted lamellar grafts, or the virus that is present does not receive the "reactivation message" because of the lack of corneal nerves and therefore cannot reactivate. Because the corneal nerves were severed by the LK approach the migration of virus from the graft to the peripheral cornea and retrograde transport to the trigeminal ganglion would be impeded. The observation period of this study was relatively short. Perhaps a longer healing time to allow corneal nerves to reinnervate the corneal graft would enable the virus to move into the trigeminal ganglion and establish latency, and subsequently be able to be reactivated.<sup>2,28,29</sup> This may explain why HSK recurs in LK patients who receive an HSV-1 latently infected corneal graft.<sup>13,27</sup>

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