Experimental disciform edema and necrotizing keratitis in the rabbit

Joseph F. Metcalf, James I. McNeill, and Herbert E. Kaufman

The development of experimental disciform edema and necrotizing keratitis in the corneas of rabbits following intrastromal inoculation with the RE strain of herpes simplex virus is described. Following an initial episode of conjunctivitis and epithelial keratitis, a mild, centrally localized, stromal edema developed on the fifth day. Stromal edema, opacification, and neovascularization of the cornea reached maximum severity on the seventh to twenty-second day, and began to fade in most eyes thereafter. On the twenty-ninth day most corneas have attained a resolved state characterized by subepithelial granular opacities. Several eyes were observed which developed central necrotizing keratitis. Marked similarities between the animal model and human herpetic stromal keratitis were apparent. Histological observations show that early necrotizing keratitis in the rabbit is characterized by an infiltration of plasma cells and lymphocytes in the limbus, with polymorphonuclear leukocytes, lymphocytes, and macrophages in the central cornea.

Key words: herpes simplex virus, animal model (rabbit), cornea (rabbit), biomicroscopy, histology, herpetic keratitis.

Herpes simplex virus (HSV) causes natural disease only in man, but a wide variety of laboratory animals and cell cultures are readily infected experimentally. The cornea of the rabbit has proved to be an excellent model system in which to study the induction of herpetic keratitis, and evaluate antiviral drugs for use in treating human patients. Application of a suspension of HSV to the corneas of rabbits results in the appearance of punctate lesions which are soon followed by the development of dendritic and geographic ulcers and edema of the epithelium. The virus may subsequently invade the stroma and endothelium of the cornea as well as the anterior chamber. The dissemination of the virus in these tissues has been described. A delayed complication of herpetic keratitis in human beings is a deep stromal disease with disciform edema. The milder form of the disease, characterized by localized edema and opacification, may persist for 2 to 3 months and then heal with...
little residual scarring. More severe forms of stromal keratitis are frequently seen with dense cheesy opacities in the corneal stroma. This type of lesion is accompanied by necrosis of stromal collagen fibers leading to the formation of permanent scarring. Vascularization of the cornea is associated with the necrotizing keratitis.13

Histologically, stromal keratitis is characterized by an infiltration of polymorphonuclear leukocytes and macrophages. This initial inflammatory reaction is replaced by an invasion of lymphocytes and plasma cells, with scattered macrophages.13

Severe uveitis, hypopyons, irregular keratic precipitates, and secondary glaucoma are complications of herpetic keratitis that may be observed during the course of the disease.12,14

Animal models of herpetic stromal keratitis have long been sought for the purpose of studying the etiology of the disease and development of an appropriate therapeutic program. Williams and associates15 described the induction of experimental disciform keratitis by several strains of HSV, but these viruses have either been lost or no longer have the capacity to induce the disease in the rabbit cornea. Stromal edema resembling that seen in herpetic keratitis has also been demonstrated in guinea pigs and rabbits following the corneal injection of soluble HSV antigens into previously sensitized animals.16,17 However, these experimental models do not show the necrotizing keratitis with destruction of corneal stroma that leads to blindness in human stromal herpes.12 Sery and associates18,19 were unsuccessful in inducing disciform edema or necrotizing keratitis with several widely divergent strains of HSV. However, Irvine and Kimura have reported that the RE strain of Herpesvirus hominis has a strong propensity for inducing stromal keratitis in rabbits without incurring a high mortality from encephalitis.

In this report we describe biomicroscopic and histological observations showing that the RE strain of HSV provides a simple, reproducible model of disciform edema and necrotizing keratitis, presenting a clinical syndrome similar to the stromal disease observed in human patients.

Materials and methods

Virus strain. The RE strain of HSV was obtained from Dr. Chandler R. Dawson (San Francisco) and stored at -70° C. Prior to use, the virus was passed through human embryonic kid-

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C = Human embryonic kidney cell lysate.
V = HSV strain RE in cell lysate.
0 = No injection.
V* = Virus suspension heated at 60° C. for 60 minutes.
Disciform edema and necrotizing keratitis

Fig. 2. Incidence and duration of epithelial keratitis, disciform edema, and necrotizing keratitis in a rabbit model.

Histology. Four additional rabbits with early necrotizing keratitis were sacrificed at 3 weeks postinfection. The corneas were fixed in 4 per cent formaldehyde in phosphate buffer, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results

Biomicroscopic observations. The un.injected corneas of animals which received virus in the contralateral eye remained clear throughout the 63 day period of observation. All of the corneas which were injected with cell suspension or heat-killed virus progressed through normally expected healing stages for an intrastromal injection, with no unusual inflammation and no permanent sequelae except small injection scars.

Observations of the corneas of animals which received intrastromal injections of the RE strain of HSV (16 eyes) are described for representative days of observation. The incidence and duration of corneal disease, without regard to severity, are shown graphically in Fig. 2. Following an initial episode of punctate and dendritic
keratitis which rapidly clear, a high incidence of localized stromal edema appears which is maintained for about 2 weeks, between the seventh and twenty-second day. Although the stromal edema resolves in most eyes, necrotizing keratitis develops in about 25 per cent of the inflamed eyes, resulting in permanent scarring and vascularization of the cornea.

Stromal edema was graded as mild if slight edema with or without early infiltration was observed; as moderate if gross clouding, edema, and infiltration were present with the pupillary border seen indistinctly; and as severe if the cornea was totally opaque, with edema and infiltration.

Day 1. The corneas were clear with little evidence of inflammation except needle tracts.

Day 3. A scant serous or mucopurulent conjunctivitis was present, and a diffuse, early (irregular), punctate keratitis with a few small dendritic figures was noted.

Day 5. A scant to moderate mucopurulent conjunctivitis was present. A moderate keratitis with a few dendritic figures, and the first mild, central stromal edema were observed.

Day 7. Both the conjunctivitis and epithelial keratitis were resolving, and central stromal edema was clearly evident in most eyes (Fig. 3).

Day 10. The punctate and dendritic keratitis was cleared, but some epithelial edema was present. A moderate to severe central stromal edema was clearly evident in most eyes. A small hypopyon was observed in one eye.

Day 12. A moderate to severe central stromal edema was present in all of the eyes except three, in which the edema was mild. A mild to moderate epithelial edema was also seen in four eyes.

Day 15. A mild to moderate stromal edema was observed. The epithelial edema was cleared. The first peripheral neovascularization was noted in three eyes. Two corneas were observed in which the stromal disease had resolved to subepithelial granular opacities.
Day 18. Three eyes were observed with advancing peripheral vascularization nearing the central third of the cornea (Fig. 4). A mild to moderate stromal edema was evident in most eyes. Two eyes with endothelial keratic precipitates were observed.

Day 22. The stromal edema appeared to be fading in most eyes, and the neovascularization was unchanged. About one-third of the eyes showed the subepithelial granular opacities characteristic of the resolving state. One eye with keratic precipitates was still present.

Day 29. Two eyes were observed with central stromal necrosis. Neovascularization extended to the necrotic area. In the remaining eyes the vascularity was stable or fading and the stromal edema was mild. About one-half of these eyes were in the resolved state with subepithelial granular opacities.

Day 35. Eyes with stromal necrosis were unchanged. The remaining eyes were in the resolved state characterized by subepithelial granular opacities in the central cornea, but were otherwise clear.

Day 63. Several eyes were clear, but most remaining eyes showed the finely granular subepithelial opacities described earlier.

An example of chronic stromal necrotizing keratitis (healed) is shown in Fig. 5.

Histological observations. The corneas of rabbits sacrificed when early stromal necrosis was present (21 days) showed a heavy accumulation of plasma cells and lymphocytes at the limbus (Fig. 6). In the central areas of the cornea two types of inflammatory reaction were found. Large subepithelial foci of necrotic stroma were found, containing a cellular infiltrate composed of a mixed population of lymphocytes and macrophages with scattered polymorphonuclear (heterophilic) leukocytes and plasma cells (Fig. 7). Other foci were found in which the infiltrating cells were almost entirely composed of polymorphonuclear leukocytes (Fig. 8). Many of these cells appeared to be in various stages of disintegration, consistent with the role of polymorphonuclear leukocytes in releasing proteolytic enzymes after death. Inflammatory cells were also found scattered throughout the stroma.
Fig. 7. Area of central stroma infiltrated with a mixed population of inflammatory cells, including lymphocytes and macrophages.

Fig. 8. Area of central stroma showing an infiltration of polymorphonuclear leukocytes.
Discussion

The biomicroscopic and histological observations described in this report indicate that injection of the RE strain of HSV into the corneal stroma of the rabbit eye leads to the development of a characteristic herpetic stromal keratitis syndrome, very similar to that described by ocular pathologists. The appearance of typical keratic endothelial precipitates and an occasional hypopyon and neovascular invasion of the cornea are all clinical symptoms observed in human patients with stromal herpes.\textsuperscript{12-14} The failure of the disease to resolve in some eyes, which became necrotic and ulcerative instead, is suggestive of necrotizing keratitis as seen in man.

The presence of heterophils, plasma cells, macrophages, and lymphocytes in the corneas of experimental animals indicates that a complex inflammatory reaction is associated with the induction of herpetic stromal keratitis in the rabbit. Further definition of histological and pathological events during the early stages of the disease, studies not possible in human patients, could provide a better understanding of the pathogenesis of this blinding disease and may lead to improved clinical management.

REFERENCES