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Prior Corneal Scarification and Injection of Immune Serum are Not Required Before Ocular HSV-1 Infection for UV-B-Induced Virus Reactivation and Recurrent Herpetic Corneal Disease in Latently Infected Mice

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Abstract

Purpose—Blinding ocular herpetic disease in humans is due to spontaneous reactivation of herpes simplex virus type 1 (HSV-1) from latency, rather than to primary acute infection. Mice latently infected with HSV-1 undergo little or no *in vivo* spontaneous reactivation with accompanying virus shedding in tears. HSV-1 reactivation can be induced in latently infected mice by several *in vivo* procedures, with UV-B-induced reactivation being one commonly used method. In the UV-B model, corneas are scarified (lightly scratched) just prior to ocular infection to increase efficiency of the primary infection and immune serum containing HSV-1 neutralizing antibodies is injected intraperitoneally (i.p.) to increase survival and decrease acute corneal damage. Since scarification can significantly alter host gene transcription in the cornea and in the trigeminal ganglia (TG; the site of HSV-1 latency) and since injection of immune serum likely modulates innate and adaptive herpes immunity, we investigated eliminating both treatments.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Material and Methods—Mice were infected with HSV-1 with or without corneal scarification and immune serum. HSV-1 reactivation and recurrent disease were induced by UV-B irradiation.

Results—When corneal scarification and immune serum were both eliminated, UV-B irradiation still induced both HSV-1 reactivation, as measured by shedding of reactivated virus in tears and herpetic eye disease, albeit at reduced levels compared to the original procedure.

Conclusion—Despite the reduced reactivation and disease, avoidance of both corneal scarification and immune serum should improve the clinical relevance of the UV-B mouse model.

Keywords

Eye; HSV-1; latency; mice; reactivation; UV-B

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is prevalent worldwide, with estimates of up to 90% of people harboring the virus.[1–4] Following a primary ocular infection, HSV-1 travels up axons to the trigeminal ganglia (TG) where it establishes lifelong latency.[1,5] Sporadic HSV-1 reactivations result in virus returning to and replicating in the cornea.[1] At this time, infectious virus can be detected in tear films by swabbing and plating the tears on monolayers of tissue culture indicator cells.[5] Individuals harboring these latent HSV-1 infections have frequent episodes of spontaneous reactivation and virus shedding in tears – as often as 3 out of every 10 days (30%).[3] Fortunately, most HSV-1 reactivations and shedding do not lead to clinical symptoms (i.e. asymptomatic shedding).[1,6–8] However, a small number of individuals are symptomatic, with frequent bouts of recurrent herpetic corneal disease, and may experience severe, vision threatening herpes stromal keratitis (HSK).[1] Approximately 450,000 adults in the US have a history of recurrent herpetic ocular disease.[2,4] A reliable animal model is critical to investigate the cellular and molecular mechanisms that regulate latency/reactivation and control recurrent herpetic disease and to test the efficacy of therapeutic vaccines and drugs against herpes reactivation and recurrent disease.

Most animal models of ocular herpes infection and disease employ either mice or rabbits.[9–12] Rabbits have the advantage of HSV-1 spontaneous reactivation with shedding of virus in tears at a rate of ~10%.[10,13,14] However, HSK is rare in this model and the currently available immunological reagents for rabbits are limited. For most virologists and immunologists, the mouse is the preferred model due to the availability of: (i) well-characterized immunological reagents to study the underlying cellular and molecular immune mechanisms that control herpes infection and disease; (ii) large numbers of inbred and transgenic strains; and (iii) specific immune molecule knockout strains.[9,12,15] However, in contrast to humans, recurrent HSV shedding and recurrent herpetic disease does not occur in mice because HSV spontaneous reactivation is either extremely rare or does not occur.[16,17] Thus, studying mechanisms that control HSV-1 reactivation and recurrent herpetic eye disease in mice requires a clinically reliable method of inducing viral reactivation that leads to HSK in a significant proportion of eyes.[18–23] Euthanizing the mouse and removing and explanting latently infected TG into tissue culture media is a

commonly used method of inducing reactivation of HSV-1, but this has the obvious disadvantage of being an *ex vivo* system with no possibility of determining virus shedding and recurrent eye disease. Thermal stress can be used to induce HSV-1 reactivation, but reactivation is usually assayed by removing TG and looking for the presence of infectious (i.e. reactivated) virus in cell free extracts.[24] There are no reports indicating that reactivation of HSV-1 by thermal stress results in recurrent corneal disease. Other methods that have been used to induce HSV-1 reactivation in mice include iontophoresis of epinephrine, cadmium, cellophane, retinoic acid, cyclophosphamide plus dexamethasone, dimethyl sulfoxide, xylene, sodium butyrate and physical restraint.[24–35] However, to the best of our knowledge, none of these methods have been reported to induce recurrent herpetic corneal disease.

In contrast, UV-B irradiation of the eyes of mice latently infected with HSV-1 induces both shedding of reactivated virus in tears and a significant amount of recurrent herpetic corneal disease.[18–23] Although the UV-B mouse model is useful for investigating the cellular and molecular mechanism involved in reactivation of HSV-1 from latently infected TG and in recurrent herpetic corneal disease, there are two potential concerns with this model. First, although the virulent HSV-1 strain McKrae is used in this model, prior to ocular infection the corneas are scarified (lightly scratched with a small gauge needle). Since the McKrae strain of HSV-1 can efficiently infect mouse corneas that have not been scarified, this appears to be a carryover from working with other HSV-1 strains that do require corneal scarification for efficient ocular infection. Since corneal scarification can alter host gene expression,[36] it would be better to avoid corneal scarification when possible. Second, to decrease both death and damage to the corneas from acute eye disease following primary HSV-1 infection, the mice are injected i.p. with immune serum containing neutralizing antibodies to HSV-1 (ImSr). This procedure alters the normal course of viral infection and would be expected to also alter the innate and adaptive herpes immune response to primary infection, and hence subsequent memory immune responses to HSV-1 following re-exposure to viral antigens (i.e. reactivated virus). Thus, it would be logical to avoid the use of immune serum so that the UV-B model would more closely reflect the natural clinical situation. We report here that UV-B-induced shedding of reactivated virus in tears and UV-B-induced recurrent herpetic disease can both be achieved without employing either corneal scarification or immune serum.

MATERIALS AND METHODS

Cell Lines

Rabbit skin (RS) cells were maintained in Eagle minimal essential medium (MEM) with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 10% fetal bovine serum (Promega Scientific, Madison, WI), penicillin (100 U/ml) and streptomycin (100 mg/ml) (Sigma, St. Louis, MO).

Viruses

HSV-1 strain McKrae was used for all studies. The virus was triple plaque purified and passaged only two or three times in rabbit skin (RS) cells prior to use as we previously described.[13]

Mice

Eight- to 10-week-old female C57BL/6 mice (Jackson Labs, Las Vegas, NV) were used in all studies. All animal studies conform to the UC Irvine IACUC guidelines and the guidelines of the US National Institute of Health.

Infection of Mice

Ocular infection of mice was performed as described[21] using 1×10^6 pfu of McKrae per eye, except that both eyes were used. Briefly, mice were anesthetized and corneas were scarified (i.e. the epithelium was lightly scratched) in a crosshatched pattern of 4–5 vertical and 4–5 horizontal scratches using a 25-gauge needle. Each mouse received an i.p. injection of 0.5 ml of pooled immune serum containing HSV-1 neutralizing antibodies with a 50% plaque reduction neutralization titer of approximately 1:128. Pooled serum from rabbits latently infected with HSV-1 was used in most experiments instead of pooled human serum used by others.[21] Primary ocular HSV-1 infection and eye disease induced following UV-B irradiation were similar with both sera (data not shown).

UV-B Irradiation of Mouse Eyes, Monitoring Shedding of Reactivated HSV-1 in Tears and Monitoring Recurrent Eye Disease

On day 30 post-infection all eyes were examined under a dissecting microscope and any eyes with significant disease were excluded. This was up to 25% of the eyes when eyes were scarified and given immune serum prior to infection and up to 10% of eyes infected without scarification or immune serum. UV-B irradiation was done following the procedure described[21] as closely as possible, except that both eyes of each mouse were used instead of just one. Mice were anesthetized and placed on a piece of cardboard on top of a TM20 Chromato-Vu transilluminator (UVP, San Gabriel, CA), which emits UV-B at a peak wavelength of 302 nm. The cardboard contained five holes, each the size of a mouse eye. Mice were positioned with one eye directly over a hole so that only the eye was exposed to the UV-B source. Each eye was irradiated with 250 mJ of UV-B light cm^2 (approximately 1 min exposure on the transilluminator). Tears were collected from each eye from day 1 to 7 post-UV-B irradiation by swabbing the eye with a moistened Dacron swab. Individual swabs were placed into 1 ml of tissue culture media, which was then plated on RS cells to detect reactivated virus as we previously described.[13,37] Recurrent eye disease was monitored at various times post-UV-B irradiation on a scale of 0–4 as described[21] by an individual masked for the mouse's experimental group. Disease was scored using a 0–4 scale indicating the percent of the corneal surface with significant clouding (0 = no disease; 1 = 25%; 2 = 50%; 3 = 75%; 4 = 100%).

RESULTS

Primary Herpetic Corneal Disease Following HSV-1 Ocular Infection of Mice

Five groups of 10 C57BL/6 mice (female, ~8 to 10 weeks old) were infected with 1×10^6 pfu/eye of HSV-1 strain McKrae, as we previously described.[38–40] This is the same strain of HSV-1 used in previous reports of UV-B-induced HSV-1 reactivation and recurrent disease. In group 1, corneas were lightly scarified (Scr) using a 25 gauge needle to make 4–5 vertical and 4–5 horizontal scratches on the corneal epithelium in a cross hatched pattern and then infected (group McKrae + Scr), as previously described.[21,41] Mice in group 2 were given a 0.5 ml i.p. injection of pooled rabbit immune serum (ImSr) having an HSV-1 neutralizing antibody titer of approximately 1:128, and corneas were scarified and infected (Group McKrae + Scr + ImSr). Mice in group 3 were infected (Group McKrae), but without prior corneal scarification or immune serum. Control groups 4 and 5 included mock-infected mice (Group Mock) and mice that were given immune serum but were not infected and whose corneas were not scarified (Group ImSr).

Following primary ocular infection, acute corneal disease was examined in all groups on days 5 and 7 post-infection (p.i.) (Figure 1). As expected, mice in group 1 whose corneas were scarified prior to infection, but who were not given immune serum, had the most severe primary infection (Figure 1; McKrae + Scr). They had the highest average corneal disease scores on day 5 and all the mice died prior to corneal disease being examined on day 7 (Figure 1B; N/A). In contrast, no mice in any of the other groups died from the primary infection. Immune serum modulated the infection and protected the mice in group 2 whose corneas were scarified prior to infection against death and significantly decreased the severity of corneal disease on day 5 ($p = 0.04$, Figure 1; McKrae + Scr + ImSr). Mice in group 3 (McKrae), which were infected without prior corneal scarification or immune serum had significant corneal disease on day 5 p.i., compared to the uninfected control groups 4 and 5 ($p = 0.02$, Figure 1A; McKrae versus Mock or versus ImSr). Mice in group 3 (McKrae) had significantly less corneal disease compared to mice in groups 1 and 2, whose corneas were scarified prior to infection (Figure 1A; McKrae versus McKrae + Scr + ImSr and McKrae versus McKrae + Scr; $p \leq 0.005$,). As expected, the Mock and ImSr control groups, which were not infected with McKrae, had no corneal disease.

On day 7, the McKrae + Scr + ImSr group had significantly more corneal clouding than the uninfected Mock and ImSr control groups (Figure 1B; $p<0.001$). The McKrae + Scr + ImSr group also appeared to have more eye disease than the McKrae group which appeared to have more corneal clouding than the Mock or ImSr groups, however, these differences did not reach statistical significance (Figure 1B; $p>0.05$ for both). Overall, the results in Figure 1 confirm the concept that when corneal scarification is used with high dose (1×10^6 pfu/eye) McKrae infection, passive neutralizing Abs (ImSr) must be employed to allow for survival of mice.[21,23]

Corneal Scarification and Immune Serum Treatment are Not Essential for UV-B-Induced Recurrent HSV-1 Disease in Mice Latently Infected with HSV-1 Strain McKrae

To determine if UV-B irradiation would induce significant amounts of recurrent corneal disease in mice that were infected with McKrae alone, without corneal scarification or immune serum, mice latently infected as in Figure 1 were irradiated with UV-B on day 31 post-infection (p.i.), as previously described[21] (Figure 2). One day prior to UV-B irradiation, all eyes were examined under a dissecting microscope and any eyes with significant corneal disease were excluded from the remaining studies (see legend to Figure 2). The UV-B source used was a TM20 Chromato-Vu transilluminator (UVP, San Gabriel, CA), which emits UV-B at a peak wavelength of 302 nm. Additional details of the UV-B model are in the "Materials and Methods" section and in the legend to Figure 2. Compared to the uninfected control group that received immune serum alone (ImSr), the McKrae + Scr + ImSr group had significantly more corneal clouding on all days from day 7 to day 30 post-UV-B irradiation (Figure 2A; $p<0.05$). The ability of UV-B irradiation to induce recurrent herpetic corneal disease is consistent with previous reports.[21,23] To determine if corneal scarification and treatment with immune serum are required for the UV-B irradiation-induced recurrent HSV-1 corneal disease, we compared the McKrae group to the Mock group (Figure 2B). Again, the corneal clouding induced in the HSV-1 latently infected mice (McKrae) was significantly higher than in the control group (Mock). Thus, UV-B irradiation can be used to induce significant recurrent eye disease in latently infected mice that were initially infected without corneal scarification or immune serum. Figure 2(C) compares the average severity of recurrent corneal disease in the McKrae group vs. the McKrae + Scr + ImSr group. The average recurrent eye disease scores in the McKrae alone group appeared lower compared to the McKrae + Scr + ImSr group. However, the differences only reached statistical significance on day 17 after UV-B irradiation. On all other days examined, there was no significant difference between the two groups.

We next compared the two uninfected control groups. Surprisingly, the ImSr control group, which received a single i.p. injection of immune serum containing HSV-1 neutralizing antibodies, had significantly more corneal clouding compared to the Mock group on every day examined after UV-B irradiation. Since neither group was infected with HSV-1, and except for the immune serum injection both groups were treated identically, it was unexpected that UV-B irradiation would result in so much corneal clouding in the ImSr group compared to the Mock group. It should be noted that in previous reports using UV-B to induce recurrent herpetic corneal disease the experimental group was equivalent to the McKrae + Scr + ImSr group in this report and the control group was typically naïve mice (i.e. no immune serum), which is equivalent to the Mock group in this report.[21,23] The results in Figure 2(D) strongly suggest that the proper control group for the McKrae + Scr + ImSr experimental group is the ImSr group (as in Figure 2A) rather than the Mock group (i.e. no ImSr). In the studies shown above anti-HSV-1 pooled rabbit immune sera was used to protect against the primary infection, while in previous reports anti-HSV-1 pooled human immune sera was usually used. When the pooled rabbit immune sera used in this study was compared to pooled human immune sera, the corneal clouding scores following UV-B irradiation were similar (*data not shown*). Thus, the herpetic eye disease induced by UV-B irradiation in other reports may have been over estimated.

Corneal Scarification and Immune Serum are Not Required for UV-B-Induced Reactivation (Shedding in Tears) of Latent HSV-1 in Mice Infected with McKrae

To determine if UV-B irradiation would induce reactivation of HSV-1 (as determined by shedding of virus in tears) in mice that were infected with McKrae without corneal scarification or immune serum, mice were infected as above with corneal scarification plus immune serum (McKrae + Scr + ImSr) or without scarification or immune serum (McKrae) (Figure 3). Thirty-one days p.i. when latency was well established, eyes were irradiated with UV-B, as described above. Tears were collected daily for 7 days, as described in the "Materials and Methods" section and in the legend to Figure 1. Tears were then plated on indicator cells to determine the presence of infectious virus indicative of viral reactivation from latently infected trigeminal ganglia (TG) and its return to the eye. Figure 3 shows the percentage of eyes in which virus was detected on at least 1 day. Virus shedding was detected in significantly more eyes from the McKrae + Scr + ImSr group compared to the McKrae group (Figure 3; $p = 0.034$). Nevertheless, UV-B irradiation clearly induced HSV-1 reactivation in mice that were infected with McKrae alone, without corneal scarification and immune serum. These results indicate that significant reactivation of HSV-1 (as determined by shedding of virus in tears) can be obtained when eyes are infected with McKrae without corneal scarification and without immune serum.

DISCUSSION

The results reported here support the usefulness of the mouse UV-B-induced reactivation model of HSV-1. They further demonstrate that prior corneal scarification (which increases the effective initial infectious dose of McKrae) plus treatment with HSV-1 immune serum (which is required to protect against death and to reduce acute eye disease when scarified corneas are infected with McKrae) are not necessary for UV-B irradiation to induce both viral reactivation and recurrent herpetic eye disease. Since both corneal scarification and injection of immune serum can distort the primary herpes infection and immunity and thus alter what happens during/following reactivation from latency, avoiding both procedures would appear to produce a more clinically relevant model. We would suggest that this is particularly germane to the investigation of cellular and molecular mechanisms that control recurrent herpetic eye disease since the amount of disease induced by UV-B irradiation was similar at most times in mice infected with or without corneal scarification plus immune serum.

The UV-B mouse model of induced HSV-1 reactivation has been used in many studies[18–23,42–48] and has produced a large amount of important information, especially regarding the host immune responses associated with recurrent herpetic disease. Unfortunately, to the best of our knowledge, all such studies used a combination of corneal scarification, just prior to ocular HSV-1 infection and i.p. injection of pooled HSV-1 immune serum just prior to or shortly after corneal infection. Corneal scarification has been shown to alter host gene expression.[36] Passive transfer of immune serum alters the natural course of the primary HSV-1 infection and thus likely modulates the initial innate and adaptive immune responses to the infection. This logically alters the host memory immune responses upon re-exposure to the virus, following reactivation from latency. Since recurrent ocular

herpetic disease (herpes stromal keratitis; HSK) is an immunopathological response, injection of immune serum contain HSV-1 neutralizing antibodies during primary infection likely alters the development of HSK. Thus, as currently employed, the ocular UV-B model of induced HSV-1 reactivation in mice may not be as predictive of the clinical situation as previously thought.

The studies reported here were undertaken in an attempt to eliminate both corneal scarification and passive immune serum treatment from the mouse UV-B model of induced HSV-1. In the course of these studies, we included an additional control group that does not seem to have been included in previous studies with the mouse UV-B model of induced HSV-1 reactivation and HSK. This control group, uninfected mice that received immune serum alone (the group labeled ImSr in Figures 1 and 2), surprisingly developed significantly more corneal clouding following UV-B irradiation compared to mock infected mice (Figure 2D) that did not receive immune serum (the group labeled Mock in Figures 1 and 2). The corneal clouding in the ImSr group could not have been caused by transfer of infectious virus in the immune serum, because HSV-1 is not disseminated in the circulation and the immune serum was heat inactivated which would have killed any virus. Since the infected mice in previous studies were given immune serum, the ImSr control group used here is a more logical control than the Mock control group to which the infected mice were previously compared. As shown in Figure 2(B), when using McKrae without corneal scarification and immune serum, the Mock group is the proper control and this avoids the high level of corneal clouding induced by UV-B irradiation of the ImSr control group.

The underlying mechanisms that lead to clouding in the cornea of uninfected mice following the passive transfer of immune serum remain to be fully determined. Among potential mechanisms are: (i) formation or deposition of Abs/Ags complexes in the cornea. This might be the result of Ags becoming sequestered in the cornea, due to a lack of corneal vascularization, and later responding to Abs from the transferred immune serum entering via the limbal circulation; (ii) formation of immune precipitates (e.g. macrophages, Langerhans cells) in the cornea triggered by passive immunization with pooled rabbit or human serum could also result in opacity in the stroma.[49] Immune precipitates can lead to clouding in the cornea, which remain visible for days and then disappear with no clinical signs of keratitis; and (iii) it is possible that heat inactivated pooled rabbit or human serum, although testing negative for infectious HSV-1, had undetected viral particles or viral Ags that trigger a yet-to-be-determined local immune response that leads to clouding in the cornea.

Our long-term goal is to delineate the cellular and molecular immune mechanisms that control virus reactivation from latently infected TG and subsequent recurrent corneal herpetic disease. This information is important for developing long-lasting antiviral drugs or immunotherapeutic strategies that successfully prevent virus reactivation and/or ameliorates recurrent ocular herpes. Results from mouse models have yielded tremendous insights into the protective and immunopathological mechanisms during primary acute versus latent infections.[50–54] However, the extrapolation of results from primary HSK and latent HSV-1 to clinical recurrent herpetic disease remains largely anecdotal. This is mainly because the immune cellular and molecular mechanisms that operate during primary acute and latent infections are in most part different from those that operate during recurrent ocular herpetic

disease.[21,55,56] Despite evidence that clinical recurrent HSK is due to virus reactivation from latency,[57,58] most pre-clinical animal studies investigating the immune mechanisms that orchestrate recurrent HSK have used primary acute infection of mice,[59–66] because spontaneous HSV-1 reactivation, virus shedding in tears, and recurrent HSK either does not occur at all or occurs at very low levels in mice.[16,17] Only a handful of pre-clinical studies have employed a mouse model of induced recurrent herpetic corneal disease.[21,56,67]

Among many methods of induced HSV-1 reactivation developed during the last four decades, the UV-B model uses corneal scarification to maximize the primary ocular infection and pooled immune serum containing HSV-1 neutralizing antibodies to protect the cornea from damage (reviewed in Ref. [[21,56,67]]). The present report reproduced this model and, in addition, eliminated both scarification and immune serum treatment. Although our methods decreased UV-B-induced reactivation and recurrent disease we feel that this is a more appropriate model to study the immunopathological mechanisms underlying clinical recurrent disease. Moreover, we have validated the UV-B light irradiation model in our novel susceptible (BALB/c genetic background) HLA transgenic (Tg) mouse model of ocular herpes by showing that a significant number of UV-B irradiated eyes developed recurrent HSK (data not shown). In our opinion, the HLA Tg mice combined with UV-B-induced recurrent disease is the best available small animal model to study the role of HLA-restricted $CD8^+$ T-cells specific to human HSV-1 epitopes in recurrent HSK.

In conclusion, three major finding were presented in this report: (i) We have shown that UV-B irradiation of the eyes of latently infected mice can successfully induce HSV-1 reactivation and recurrent herpetic disease without the need for corneal scarification and passive immune serum treatment at the time of the initial ocular infection; (ii) naïve mice are not the best control for studies in which the infected group is treated with passive immune serum. This is because following UV-B irradiation, the more proper control group (given immune serum) developed significantly more corneal clouding than the naïve mouse control group; and (iii) mice infected with McKrae without corneal scarification or immune serum appeared to develop more eye disease following UV-B irradiation compared to their Mock control group than did mice infected following corneal scarification and immune serum treatment compared to their proper immune serum control group (compare Figure 1A and B). Thus, elimination of corneal scarification and passive immune serum from the UV-B model may not only make the model more similar to the clinical situation, but may also result in increased recurrent eye disease relative to controls.

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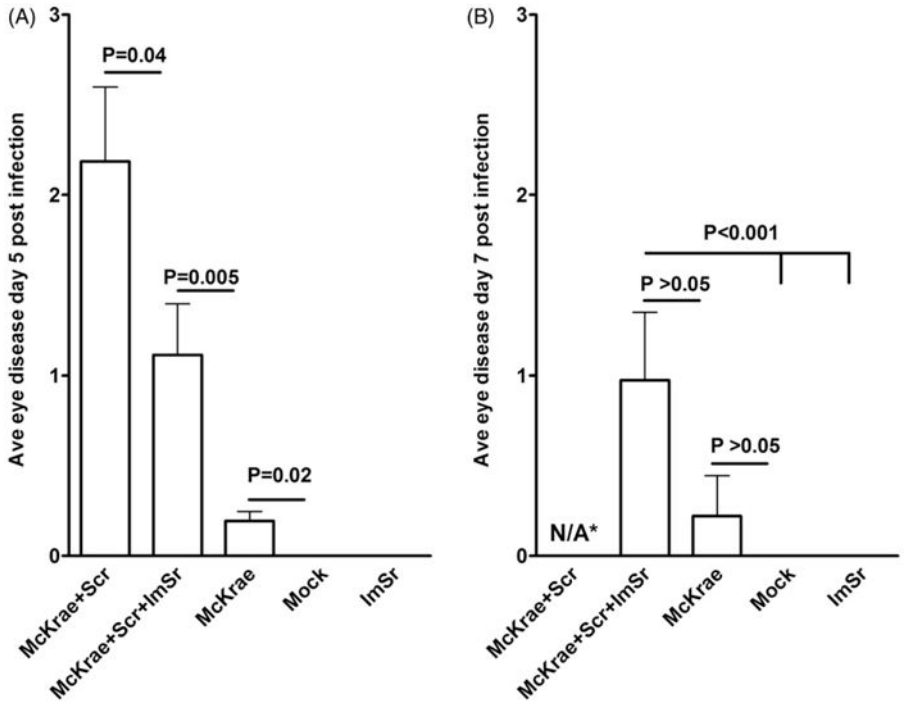

FIGURE 1.

Comparison of acute herpetic eye disease induced by McKrae with and without corneal scarification and/or i.p. immune serum, versus McKrae alone. Ocular infection was performed with or without prior corneal scarification and with or without i.p. injection of anti-HSV-1 immune serum as described in the “Materials and Methods” section. Each bar shows the mean ± SE of 20 eyes. Groups (bars): McKrae + Scr (corneal scarification prior to infection); McKrae + Scr + ImSr (corneal scarification and serum prior to infection); McKrae (infected with no corneal scarification and no immune serum); Mock (naïve mice); ImSr (immune serum, no virus, no corneal scarification). On day 5, post-infection the McKrae + Scr group had significantly more eye disease than any other group, the McKrae + Scr + ImSr group had significantly more eye disease than the McKrae, Mock or ImSr groups, the McKrae group had more eye disease than the Mock or ImSr groups, and the mock and ImSr groups had no disease. On day 7, the McKrae + Scr + ImSr group had significantly more eye disease than the Mock and ImSr alone controls and appeared to have more eye disease than the McKrae group, but this difference did not reach statistical significance. N/A indicates that the eye disease score for the McKrae + Scr group on day 7 post-infection was not applicable because all the mice in this group died between the time eyes were read on day 5 and the time eyes were read on day 7. p values were determined by Students t-test. $p<0.05$ is considered significant.

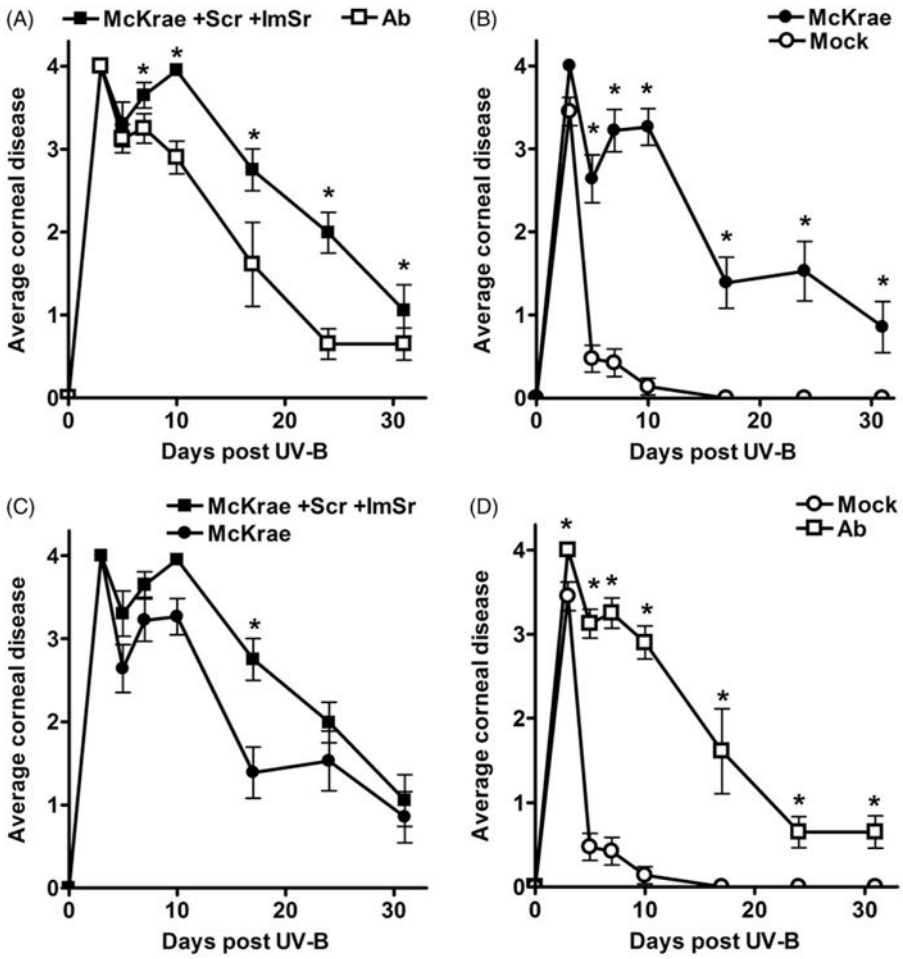

FIGURE 2.

UV-B irradiation induced significant amounts of recurrent corneal disease in eyes infected with McKrae without corneal scarification or immune serum. Groups of mice infected as described in the legend to Figure 1 were UV-B irradiated in both eyes and recurrent eye disease scored as described in the “Materials and Methods” section. Groups are as described in the legend for Figure 1. The data shown are the combined results of two separate experiments. Each time point for each group represents the mean ± SE of between 15 and 30 eyes.

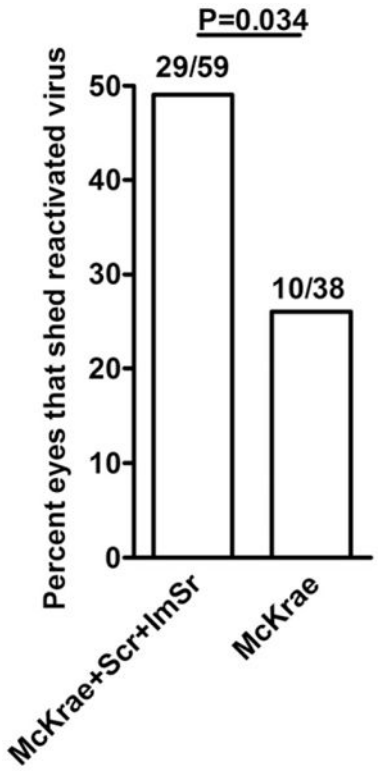

FIGURE 3.

UV-B induced virus shedding in eyes from mice latently infected with McKrae without corneal scarification and immune serum. Eyes were infected and irradiated with UV-B as described in the legends to Figures 1 and 2. Tears were collected from all eyes on days 3, 4, 5, 6 and 7 post-UV-B irradiation and plated on indicator cells (RS cells) to detect the presence of infectious virus indicative of HSV-1 reactivation as we have previously described for rabbits[13]. No virus was detected in tears prior to UV-B irradiation (data not shown). The data shown are pooled from two experiments that had similar results.