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Review Article

Rabbit and Mouse Models of HSV-1 Latency, Reactivation, and Recurrent Eye Diseases

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The exact mechanisms of HSV-1 establishment, maintenance, latency, reactivation, and also the courses of recurrent ocular infections remain a mystery. Comprehensive understanding of the HSV-1 disease process could lead to prevention of HSV-1 acute infection, reactivation, and more effective treatments of recurrent ocular disease. Animal models have been used for over sixty years to investigate our concepts and hypotheses of HSV-1 diseases. In this paper we present descriptions and examples of rabbit and mouse eye models of HSV-1 latency, reactivation, and recurrent diseases. We summarize studies in animal models of spontaneous and induced HSV-1 reactivation and recurrent disease. Numerous stimuli that induce reactivation in mice and rabbits are described, as well as factors that inhibit viral reactivation from latency. The key features, advantages, and disadvantages of the mouse and rabbit models in relation to the study of ocular HSV-1 are discussed. This paper is pertinent but not intended to be all inclusive. We will give examples of key papers that have reported novel discoveries related to the review topics.

1. Introduction

HSV-1 is remarkable in its ability to establish a lifelong latent infection in human hosts following exposure to the virus [1, 2]. Numerous animal models have been used to study the phenomenon of HSV-1 latency, particularly in relation to the specific characteristics of virus strain and genetics, host response, and environmental factors. This paper will provide summaries and examples of latency, reactivation, inhibition of reactivation, and recurrent herpetic ocular lesions in rabbits and mice.

After initial infection, HSV-1 travels by retrograde axonal transport to sensory ganglia to establish latency. In this paper, we define latency as the retention of the quiescent viral genome in host neuronal tissue, with no demonstrable infectious virus present. Latency of HSV-1 occurs in the superior cervical ganglion (SCG) and trigeminal ganglion (TG), ciliary ganglion, nodose ganglion, otic ganglion, pterygopalatine ganglion, submandibular ganglion, geniculate ganglion [1, 3], ophthalmic branch of the trigeminal nerve, and root entry zone of the trigeminal nerve into the brainstem [4–6]. Latent HSV-1 has also been described

in other nonneuronal tissues, such as the cornea [7]. Reactivation can occur spontaneously with virus strains known to be high phenotypic reactivators (HPRs), or after induction in latently infected animals [8–10]. Reactivation is identified by detection of HSV-1 DNA in tears and saliva and when infectious virus can be confirmed [1, 2]. Recurrence is when HSV-specific corneal lesions are identified during latency. Recurrent lesions occur when HSV-1 is carried by anterograde transport to initial sites of infection or any site innervated by the latently infected ganglia and results in HSV-specific lesions followed by complications, such as stromal scarring, thinning, opacity, and neovascularization [1, 2].

2. HSV-1 Strains

Each HSV strain is defined as a clinical isolate that has been plaque purified and specifically identified in a publication. In addition to animal strain and gender, the strain of the virus can influence the susceptibility to infection by HSV-1. HSV-1 strains such as McKrae and 17Syn+ have been found to exhibit a high rate of spontaneous shedding and are known as high phenotypic reactivators (HPRs) [1, 2, 8]. Low phenotypic reactivators (LPRs) exhibit a very low rate of spontaneous shedding and include F, KOS, SC-16, Rodanus, McIntyre, and CGA-3 [1, 2, 8]. One important viral recombinant LPR is LAT-negative 17ΔPst recombinant, generated by deleting LAT promoter 17Syn+ (202 bp) [11]; because both viruses originate from the strain 17 background, they are useful in studying genotypic and phenotypic characteristics within a viral strain. Sawtell et al. [12] found that of the three HSV-1 strains commonly used, KOS, 17Syn+, and McKrae, KOS differs significantly in the capacity to reactivate from latency when induced *in vivo*. They reported that neurons in ganglia infected with HSV-1 strain KOS contained significantly fewer HSV-1 DNA copy numbers than those infected with 17Syn+ or McKrae [12]. In another report with HPR strains, a higher number of latent HSV-1 DNA copies were present in the individual neurons of ganglia, which overwhelm the cellular mechanisms that silence virus transcription and promote reactivation [13]. For example, strains 17Syn+ and McKrae have been reported to establish more genome copies per neuron than KOS [14]. In one experiment, researchers used W strain cultured from a patient with typical HSV-1 dendritic keratitis and found that W strain is comparable to McKrae in its ease of induced reactivation and ocular shedding in rabbit and mouse models [15]. However, W strain is less neurovirulent than McKrae and therefore results in fewer cases of encephalitis and death [15].

The specific HSV-1 viral strain can make a significant difference in results and conclusions when studying HSV-1 latency, reactivation, and recurrent disease. For example, Hill et al. [8] studied 10 groups of rabbits latently infected with various strains of HSV-1. Viruses used could be separated into four separate reactivation frequency groups: Macintyre and CGA-3 strains showed very low spontaneous and no induced reactivation. Strain SC16 showed modest spontaneous and induced reactivation. Rodanus, RE, F, and KOS strains showed spontaneous reactivation but low induced reactivation in rabbits. HPR strains McKrae, 17Syn+, and E-43 strains showed both spontaneous and induced reactivation [8].

3. Animal Species

Mice and rabbits are used more than any other model to study HSV-1 latency, reactivation, and recurrence. Several characteristics make mouse models excellent candidates for studying HSV-1. In mice, inbred strains and transgenic strains are readily available, which has led to the widespread use of mouse models for studying ocular HSV-1. There are several thousand strains of transgenic mice, and most are named for the gene of interest which has been altered. The most common type of transgenic mouse used in the study of HSV-1 is the knockout mouse, where the activity of a single gene can be removed and its function analyzed. Table 1 summarizes reports of transgenic mice that have been used in the study of HSV-1 latency, reactivation, and recurrent disease. Many of the knockouts used for ocular herpes involved removing immune system components and are used for studying the immune system's effects on latency and reactivation. These transgenic mouse models have provided insight into the role of specific genes and cytokines involved in HSV-1 ocular disease.

Other advantages of the mouse model include its small size, which significantly reduces the amounts of drugs or chemicals needed for experiments. The low cost of boarding, as well as the relatively low cost of inbred strains, also contributes to the usefulness of mice. However, the disadvantages of mouse models primarily include the difficulty in assessing their corneal lesions and their small amounts of tissue recovered (harvested) for assays. The small tear film volume of mice decreases the efficiency of detecting infectious HSV-1 and HSV-1 DNA [1, 2, 16, 17]. The rate of spontaneous shedding of HSV-1 DNA in mice is extremely low, and there are no known reports of spontaneous recurrent lesions in immunocompetent mice.

The genetic makeup of the mouse influences susceptibility to HSV-1 latency, reactivation, and recurrent disease. Mice are available as noninbred or inbred (genetically homogenous) strains. Noninbred mice are less expensive and include the ICR, Swiss Webster, and NIH lines. Inbred strains of mice are used because of their genetic consistency and include C57BL/6 and BALB/c [17]. The strain of HSV-1, mouse strain, and mouse gender result in variability in the mortality, severity of infection, and frequency of reactivation [1, 2, 16]. For example, most strains of HSV-1 reactivate more readily in inbred BALB/c mice than inbred C57BL/6 mice, which is thought to be caused by differences in the host mouse immune system [1, 18, 19].

Rabbits also have specific advantages and disadvantages as models for studying ocular HSV-1. Rabbits have larger eyes to examine and corneal lesions easily accessible for imaging and quantification by slit-lamp examination (SLE). Spontaneous lesions occur often in rabbits latent with HPR strains of HSV-1. Their large size provides more ocular and

Table 1: Selected transgenic mice used for the study of ocular HSV-1.

Mouse name	Use of model	Author, year (reference no.)
Rag2 (−/−)	Study of ICP0 and ICP34.5	Halford et al., 1996 [102]
Stat1 (−/−)	IFN response to viral infection	Pasieka et al., 2011 [103]
INF$\alpha\beta\gamma$R (−/−)	IFN response to viral infection	Pasieka et al., 2011[103]
IRF-3 (−/−)	Viral induction of type I INF cascade	Menachery and Leib 2009 [36]
Human ApoE 4 (+/+)	ApoE4 role in microglia immune response	Vitek et al., 2009 [104]
Stat1 (−/−)	Enhanced pathogenesis of Stat1 deficient mice	Pasieka et al., 2008 [105]
Human ApoE3 (+/+)	Human ApoE4 role in ocular herpes pathogenesis	Bhattacharjee et al., 2008 [25]
Human ApoE4 (+/+)	Human ApoE4 role in ocular herpes pathogenesis	Bhattacharjee et al., 2008 [25]
p19 (−/−)	IL-23 role in the severity of HSV-1 ocular lesions	Kim et al., 2008 [106]
ISG15 (−/−)	ISG15 role in host response to viral infection	Lenschow et al., 2007 [107]
Stat4 (−/−)	Cytokine involvement in HSV-1 stromal keratitis events	Banerjee et al., 2007 [108]
IL-1ra (−/−)	IL-1 role in HSV-1 stromal keratitis	Biswas et al., 2004 [109]
scid or rag2 (−/−)	ICP0 role in viral replication	Halford et al., 2006 [110]
wt and p53 (−/−)	Role of p43 in HSV-1 replication	Haenchen et al., 2010 [111]

neural tissues for assessment, and their abundant tear film allows easy collection of tears. However, when compared to the mouse model, inbred strains of rabbits are very expensive and difficult to obtain. Because of their larger size compared to mice, purchasing and boarding expenses for rabbits lead to their much higher costs. Inoculation of rabbits with HPR strains results in 50% mortality, and corneal inoculation with HPR strains results in stromal opacity in 5–10% of corneas [1, 2, 16, 20].

Rabbit strains that can be used for studies of ocular herpes include the New Zealand White (NZW), the Dutch Belted, and other pigmented rabbits. Rabbits with nonpigmented eyes (NZW) are the usual choice for vision research. Most strains of HSV-1 will infect any of the above rabbits, and their HSV-1 infection is more prototypical of human disease than that of mice [17]; rabbits latent with HPR strains have a high rate of spontaneous HSV-1 shedding, and their lesions share similar characteristics with human HSV-1 lesions [20].

Transgenic rabbits are very limited; however, researchers have obtained them for studying HSV-1. Chentoufi et al. [20] introduced a humanized HLA-A∗0201 transgenic rabbit model in their search for a vaccine against primary ocular herpes infection. The rabbit produces human HLA-restricted and specific T-cell responses for studying human CD8+ T-cell epitope-based vaccines. When this transgenic rabbit model is immunized with the vaccine, it produces HSV-1-specific CD8 T cells and displays reduced HSV-1 recurrent disease after induction from latent HSV-1 infection. Because the rabbit model is more similar to humans than to transgenic mice models previously used for this purpose, this transgenic rabbit could become an excellent animal model for studying these vaccines [20].

4. Mice and HSV-1 Latency

Mouse models have been used extensively to study HSV-1 latency. Latency has been described molecularly as the state in which detectable viral gene expression is limited to the abundant transcription of LAT, a region of the HSV-1 genome that encodes an 8.5 kb polyadenylated RNA, which is then spliced into introns that accumulate in the nucleus (Figure 1) [1, 2, 21]. LAT is the only viral gene abundantly transcribed during HSV-1 neuronal latency [21, 22]. The full function of LAT remains a mystery, and mouse models have been used for years to investigate its purpose. Thompson and Sawtell [23] used a LAT-deletion mutant HSV-1 virus derived from a KOS/M parent in a murine model to show that the presence of LAT increases the number of neurons in which latency is established, but LAT has not been demonstrated to be required for establishment of latency [23]. In another mouse eye model, Thompson and Sawtell concluded that LAT promotes neuronal survival in latently infected TG [24].

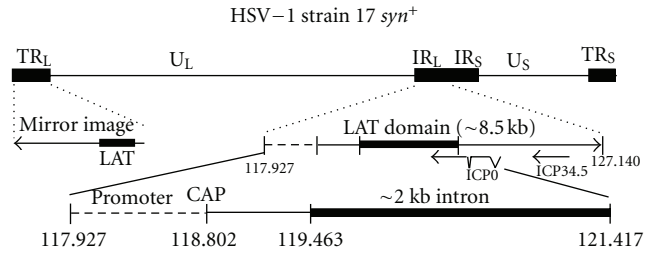

Figure 1: This figure depicts the linear HSV-1 strains 17 Syn+ noting specific regions of the genome. The abbreviations are as follows: TR_L—terminal repeat long; TR_S—terminal repeat short; U_L—unique long; U_S—unique short; IR_L—internal repeat long; IR_S—internal repeat short. Note that LAT is in two regions of the HSV-1 genome (TR_L and IR_L). An 8.5 kb polyadenylated precursor is transcribed. Within the last domain are two very important immediate-early genes, ICP0 and ICP34.5. Also note the promoter, the CAP site, and a 2.0 kb intron. Further modification results in a 1.5 kb smaller intron.

Host genetic makeup also plays a role in establishment of HSV-1 latency. Studies of ApoE have found that this gene influences HSV-1 infections [25, 26]. Three major

human alleles of the human ApoE gene code for six different isoforms of this glycosylated protein. The mouse has only one ApoE allele, which is suggested to be similar to one of the human isoforms, ApoE4. Several studies with mouse models have shown that human ApoE4 results in high neuroinvasiveness of HSV-1 [27–29]. Bhattacharjee et al. [29] studied ApoE4 with wild-type C57BL/6 mice and C57BL/6 knockout mice using corneal inoculation with HPR HSV-1 strain 17Syn+. They found that ApoE knockout mice are resistant to the neurovirulence of HSV-1 strain 17Syn+, and the wild-type mouse had a higher latent load of virus in the TG than the knockout mice. The results showed that host ApoE genetics have a significant effect on establishment of HSV-1 latent infection in mice [29]. Furthermore, Burgos et al. [27] reported in their study of transgenic mice that their ApoE4 homozygote mouse had higher viral loads of HSV-1 in the midbrain, ventricles, cortex, and cerebellum than heterozygote or null mutants after hematogenous inoculation (intraperitoneal injection). The homozygote mice also had higher viral loads of HSV-1 DNA in their TG and spinal cords, indicating that the amount of ApoE is directly linked to the invasiveness of HSV-1 in the brain [27].

Mouse models have been used to study HSV-1 nonneuronal latency. This concept was first proposed by T. J. Hill et al. [30], who isolated infectious HSV-1 from 8% (7 of 88) of ears of latently infected, nonstimulated, noninbred mice. HSV-1 DNA has been found in corneas of patients with chronic herpetic keratitis and in eye bank corneas [7]. HSV-1 could remain latent in corneas, but this has been difficult to confirm because of difficulty in proving that the virus remains latent in the cornea, rather than using the cornea as a transient site [7]. Mouse models have been used to study HSV-1 corneal shedding and the possibility of corneal latency. Abghari and Stulting [31] isolated HSV-1 from the eyes of latently infected mice and reported that infectious HSV-1 was detected earlier from TG cultures than corneal cultures. The results could suggest that the virus was latent in the cornea, because persistent viral shedding or persistent infections in the corneas would exhibit culture growth at a faster rate [31].

Mouse models provide evidence against HSV-1 corneal latency. Easty et al. [32] reported a study in which NIH mice were inoculated with HSV-1 in the cornea, and HSV-1 did not persist in the anterior segment. However, when mice were inoculated in the snout, infectious virus was detected in 40% (8/20) of corneas of noninbred latently infected mice [32]. These results suggested that either the sensory ganglion is required to establish latency, or the virus was actually reactivated from the ganglion [32]. Further study of corneal latency in animal models could lead to more efficient treatment of herpetic keratitis.

Most recently, mouse models have been used to study the role of T-cell exhaustion in controlling HSV-1 latency [33–35]. For example, Allen et al. [33] studied the role of LAT in CD8+ T cell exhaustion in the TG of mice latently infected mice. Reddy et al. [35] studied the TG in mice latently infected with HSV-1 and found that the Tim-3 (+) CD8 T cell interaction with ligand galectin-9 could play a significant role in the outcome of HSV-1 reactivation from latency. More research in these mouse models could set the stage for new therapeutic treatments to inhibit HSV-1 reactivation from latency.

4.1. Spontaneous HSV-1 Reactivation in Mice. Spontaneous reactivation of latent HSV-1 has been shown to occur in humans [1, 36–39] and in experimentally infected rabbits [1, 2, 8, 40, 41], but reports of spontaneous shedding in mice are limited (Table 2). This feature could provide an advantage over the rabbit model, as it decreases the potential complications that arise when experimentally induced shedding is difficult to dissociate from spontaneous shedding [42]. Many investigators have used the mouse model to study latent HSV-1, because they are able to assume that features of viral gene expression are attributable to latency, and not to spontaneous reactivation [43]. Many episodes of HSV-1 DNA shedding in humans are spontaneous, and it is believed that viral reactivation in the rabbit could be a more useful model for human disease for this reason [37]. However, latent mice that have been shown to spontaneously shed HSV-1 at low frequencies could indicate that experimental murine HSV-1 infection is similar to the infection in other animals and humans (see Table 2). For example, Margolis et al. provided evidence that latent mice exhibited spontaneous molecular reactivation occurring in very low frequency in their TG [16].

Table 2 represents data that demonstrate that spontaneous reactivation of HSV-1 occurs in a very low percentage of noninduced latent mice. The table summarizes data of spontaneous shedding of HSV-1, and includes the gender and strain of mouse, HSV-1 strain, tissue studied, and frequencies of detection of infectious virus. Studies have suggested that the strain and gender of mice affect the frequency of HSV-1 reactivation [18]. Similarly, the viral strain of HSV-1 affects the latent viral load within ganglia, subsequently affecting the frequency of reactivation [12]. Regardless of the difference in parameters between studies shown in Table 2, the report of spontaneous reactivation of HSV-1 in mice exhibits frequencies of 3% (3/97) to 27% (6/22). We conclude from these data that mice spontaneously shed HSV-1, but at frequencies lower than rabbits or humans.

Willey et al. [44] studied latently infected mice that had not been subjected to induced reactivation, and they showed that in 3% (3/97) of tear film swabs of the mice studied, HSV-1 infectious virus was detected. Productive cycle viral gene ICP-4 is expressed in a small number of latently infected murine TG in the absence of detectable infectious virus in their ganglia [43]. The study also noted that the neurons showing gene expression were surrounded by inflammatory cell infiltrates, suggesting recognition of the virus by the murine immune system [43]. Gebhardt et al. also showed the same immunorecognition in the rabbit eye model [17, 45].

Sawtell [46] developed an assay to quantify the number of neurons that were undergoing reactivation within murine TG during latency. Without induction, a greater percentage of HSV-1 protein-positive neurons undergoing spontaneous

Table 2: Detection of infectious HSV-1 from eye swabs—spontaneous shedding in noninduced mice.

Mouse strain	Gender	HSV-1 Strain	Tissue/fluid/identification procedure	Frequency of detection of reactivation	Authors, year (reference no.)
BALB/c (inbred)	Male	McKrae	Ocular swabs	3/97 (3%)	Willey et al., 1984 [44]; Laycock et al., 1991 [112]
ICR (Harlem outbred)	Male	Patton	Explant TG	6/22 (27%); 14 hr time period	Pesola et al., 2005 [113]
ICR (Harlem)	Male	KOS	Explant TG	1/20 (5%); 2 hr time period	Pesola et al., 2005 [113]
ICR (Harlem)	Male	KOS	Explant TG	4/18 (22%); 14 hr time period	Pesola et al., 2005 [113]
NIH-OLA (inbred)	NA	McKrae	Eye wash	1/11 (9%)	Shimeld et al., 1990 [42]
Swiss Webster (outbred)	Male	17 Syn+	TG homogenized	1/23 (4%); PI day 31	Sawtell 2003 [46]
Swiss Webster (outbred)	Male	17 Syn+	TG homogenized	1/16 (6%); PI day 60	Sawtell 2003 [46]

NA: not Available and PI: postinoculation.

reactivation were present at times most proximal to corneal inoculation. This suggests that the level of spontaneous reactivation decreases as the time postinoculation increases (Table 2) [46].

4.2. Mouse Ocular Models of Induced Reactivation. Studies of latency and reactivation are difficult to correlate in mice compared with humans, because different mouse strains have shown variable responses to different HSV-1 strains [1, 2, 7, 17, 22]. Studies have suggested that the mouse model of latency and reactivation in regard to LAT is mouse-strain dependent (Figure 1) [22]. This is unlike the rabbit ocular model, in which LAT null mutants have exhibited consistently reduced reactivation *in vivo* [11, 47]. For example, LAT null mutants have been known to reactivate poorly in some mouse models, while some reports show that LAT null mutants show normal reactivation in other mouse phenotypes [22]. In one report, BALB/c and Swiss Webster mice were infected with LAT-negative or LAT-positive virus and were assessed by explant-induced reactivation of their TG. LAT-negative virus strains reactivated poorly in Swiss Webster mice, while the LAT-negative and LAT-positive strains showed no significant difference in reactivation frequencies in BALB/c mice [22]. The genetic component in BALB/c mice that could account for this effect has not been determined.

There have been reports of LAT mutant experiments in mice that have different outcomes than similar experiments in rabbits. For example, Perng et al. [48] reported that the LAT regulation of HSV-1 virulence and reactivation could be species specific. A LAT-negative transcript that showed a lower rate of reactivation in rabbits produced increased virulence in mice. Another LAT deletion transcript that had increased virulence in rabbits had decreased virulence in mice. This study suggests that LAT could have multiple functions, accounting for the different increases and decreases in virulence in the mouse and the rabbit [48]. The authors concluded that in addition to being involved in the HSV-1 latency and reactivation cycle, LAT also plays a role in neurovirulence. Murine latent HSV-1 infection following corneal inoculation has been induced to study reactivation with several stimuli, including heat stress, cyclophosphamide (Cx) and dexamethasone (Dx), iontophoresis of epinephrine, and UVB. Table 3 cites reports of induced shedding in mice models, and the strain and gender of mouse, strain of virus, and method of induction are shown. There is evidence that mouse models can be used to induce HSV-1 shedding experimentally; the data show HSV-1 detected in ocular swabs after induction over a range of 5% (1/20) to 100% (12/12). Willey et al. [44] used transcorneal iontophoresis of epinephrine to significantly increase the incidence of ocular HSV-1 shedding in the BALB/c mouse eye model. Shimeld et al. [42] used immunosuppressants followed by UVB in a murine model to induce viral replication and found that immunosuppression in combination with UVB produced a higher incidence of both shedding and recurrent disease compared to immunosuppression alone. In another experiment, Dx and Cx were used successfully for induction without UVB, to induce shedding without the ocular trauma that occurs with epinephrine iontophoresis or UVB exposure [49]. Intraperitoneal administration of sodium butyrate, a histone deacetylase inhibitor, has also been used successfully to induce HSV-1 shedding in mice. Neumann et al. [50] have used this induction method in their study of epigenetic changes within the HSV-1 associated chromatin during latency and reactivation. The method causes changes in histone acetylation of the LAT promoter region of HSV-1 in latent TG. Treatment with sodium butyrate consistently and reproducibly induces viral reactivation with HPR strains 17Syn+ and McKrae [51]. Clement et al. [52] used intraperitoneal administration of sodium butyrate in their study of epigenetic changes and host gene expression during reactivation from latency and reported multiple roles of the LAT-ICP0 locus in viral induction. Hyperthermia has been used in mice to induce HSV-1 shedding (see Table 3). Sawtell and Thompson [53] used Swiss Webster mice and reported that immersion in 42°C water induced reactivation of HSV-1 that occurred in neurons 14 hours after hyperthermic stress.

Furthermore, Clement et al. [54] have used hyperthermic stress in HSV-1 latently infected BALB/c mice to study host gene expression and reported upregulation of mainly immunity genes, indicating a role for the LAT-ICP0 locus in induction of the body's defense during viral reactivation from latency.

Table 3: Detection of infectious HSV-1 from latent mice following stimulation.

Mouse strain	Gender	HSV-1 strain	Tissue/fluid/identification procedure	Frequency of detection of reactivation	Type or method of induction	Use of model	Authors, year (reference no.)
BALB/c (inbred)	Male	McKrae	Eye swabs	16/23 (70%)	Transcorneal epinephrine iontophoresis	Reactivation	Willey et al., 1984 [44]
NIH-OLA (inbred)	NA	McKrae	Eye wash	2/2 (100%)	Immunosuppressant	Reactivation antigen studies	Shimeld et al., 1990 [42]
NIH-OLA (inbred)	NA	McKrae	Eye wash	9/9 (100%)	Immunosuppressant UV-B	Reactivation antigen studies	Shimeld et al., 1990 [42]
NIH Swiss Webster	Male	17 Syn+	Eye swab	9/20 (45%)	Cx and Dx	LAT facilitates reactivation	Cook et al., 1991 [49]
NIH Swiss Webster	Male	XC-20, (LAT+)	Eye swab	6/19 (31.6%)	Cx and Dx	LAT facilitates reactivation	Cook et al., 1991[49]
NIH Swiss Webster	Male	X10-13 (LAT−)	Eye swab	1/20 (5%)	Cx and Dx	LAT facilitates reactivation	Cook et al., 1991[49]
BALB/c	Female	McKrae	Eye swab	15/28 (54%)	NaB in PBS	*In vivo* HSV-1 reactivation	Neumann et al., 2007 [51]
BALB/c	Female	17 Syn+	Eye swab	21/65 (32%)	NaB in PBS	*In vivo* HSV-1 reactivation	Neumann et al., 2007 [51]

Cx: cyclophosphamide, Dx: dexamethasone, LAT: latency-associated transcripts, NA: not available, NaB: sodium butyrate, and PBS: phosphate-balanced saline.

Table 4: Inhibition of induced HSV-1 reactivation in latent mice after stimulation.

Mouse strain	Gender	HSV-1 strain	Inhibitor	Frequency of detection with inhibitor	Frequency of detection without inhibitor	Type or method of induction	Authors, year (Reference no.)
BALB/c	NA	McKrae	Propranolol	12/37 (32%)	20/31 (65%)	Hyperthermia	Gebhardt and Kaufman, 1995 [62]
BALB/c	Female	McKrae	Anti-IL-6 antibodies	6/20 (30%)	14/20 (70%)	Hyperthermia	Kriesel et al., 1997 [114]
BALB/c	Female	McKrae	Anti-IL-6 antibodies	2/20 (20%)	6/8 (75%)	UVB	Kriesel et al., 1997 [114]
BALB/c	Female	McKrae	ACV	20/50 (40%)	39/50 (78%)	Hyperthermia	Gebhardt et al., 2004 [61]
BALB/c	NA	McKrae	ASA	5/36 (14%)	11/36 (31%)	Hyperthermia	Gebhardt et al., 2004 [56]
BALB/c	Female	McKrae	Celecoxib	11/47 (23%)	28/52 (54%)	Hyperthermia	Gebhardt et al., 2005 [115]
BALB/c	Female	McKrae	Bromfenac eye drops	10/24 (41.7%)	16/22 (72.2%)	Cx, Dx & hyperthermia	Higaki et al., 2009 [58]
BALB/c	NA	W strain	Alpha blockers	2/34 (6%)	10/39 (29%)	Epinephrine iontophoresis	Gordon et al., 1990 [90]

ACV: acycloguanosine, ASA: aspirin, and NA: not available.

4.3. Inhibition of Reactivation in the Mouse Model as a Tool of Therapeutic Intervention Studies. Mouse models have been useful for studying the reduction of HSV-1 reactivation (Table 4). The topical use of latanoprost in the treatment of glaucoma has been associated with viral reactivation and recurrence, and there have been reports of increased corneal epithelial lesions in humans and rabbits exposed to this prostaglandin analog [55]. These reports led to an interest in the role of prostaglandins in inducing HSV-1 reactivation [56]. Prostaglandins activate adenylate cyclase, which increases intracellular cAMP. Binding of cAMP to protein kinase A phosphorylates transcription factors, including the cAMP-responsive binding site of the LAT promoter region (Figure 1). Studies in mice have shown decreased reactivation of HSV-1 with mutations in the cAMP-responsive binding site [57].

The prostaglandin synthesis pathway is an intermediate pathway in the process of HSV-1 reactivation [56], and mouse models have been used to show that cyclooxygenase 2 inhibitors could be useful in the prevention of HSV-1 reactivation. Gebhardt et al. [56] reported that after hyperthermic stress, latently infected mice treated with celecoxib had less infectious virus on their ocular surfaces than controls (see Table 4). Higaki et al. [58] reported that bromfenac sodium eye drops significantly reduced HSV-1 ocular shedding in mice induced by immunosuppression and hyperthermia. They concluded that bromfenac sodium eye drops could be a potent medication for suppressing HSV-1 reactivation [58]. In another mouse study, acetylsalicylic acid, a nonspecific inhibitor of cyclooxygenase, reduced shedding of HSV-1 in the tears of induced mice. The authors concluded that acetylsalicylic acid suppressed viral reactivation in mice [56].

Table 5: Spontaneous shedding of infectious HSV-1 in latent rabbits.

HSV-1 strain	Tissue/fluid/identification/procedure	Frequency of detection of reactivation	Authors, year (reference no.)
Rodanus	Ocular swabs	31/40 (77%) between 31 and 100 days PI	Laibson and Kibrick,1969 [67]
McKrae	Ocular swabs	13/20 (65%)	Nesburn et al., 1967 [68]
McKrae	Ocular swabs	19/20 (95%)	Kwon et al., 1981 [9]
McKrae	Ocular swabs	2/10 (20%)	Kwon et al., 1982 [73]
McKrae	Ocular swabs	3/20 (15%)	Hill et al., 1983 [74]
Rodanus	Ocular swabs	1/3	Laibson and Kibrick,1966 [75]
McKrae	Ocular swabs	4/68 (5.8%)	Hill et al., 1987 [81]
McKrae	Ocular swabs	6/140 (4%)	Haruta et al., 1987 [10]
McKrae	Ocular swabs	28/216 (13%)	Kaufman et al., 1996 [80]
17 Syn+	Ocular swabs	31/264 (11.7%)	Kaufman et al., 1996 [80]
McKrae	Ocular swabs	10/610 (1.6%)	Beyer et al., 1989 [83]
McKrae	Ocular swabs	4/14(29%)	Beyer et al., 1990 [84]
W Strain	Ocular swabs	11/16 (69%)	Gordon et al., 2003 [15]
McKrae	Ocular swabs	14/20 (70%)	Myles et al., 2003 [86]
McKrae	Ocular swabs	6/16 (38%)	Hill et al., 1997 [87]
McKrae	Ocular swabs	13/20 (65%)	Myles et al., 2004 [89]

Chronic oral administration of antiherpetics reduces the rate of ocular, genital, and oral recurrent lesions in humans [1, 2, 37, 59, 60]. Acycloguanosine (ACV) has been proven effective for preventing recurrent HSV-2 lesions of the genital and oral labia, in human and experimental models. Gebhardt et al. [61] found that ACV treatment significantly decreased the frequency of infectious HSV-1 virus in the ocular tear film and in the cornea of latently infected mice induced by hyperthermia. The mice did not, however, show any changes in HSV-1 DNA in their TG compared with controls. ACV was effective in decreasing viral replication in peripheral tissues, but is not effective in inhibiting viral reactivation and DNA synthesis in the peripheral nervous system [61].

Propranolol suppressed latent HSV-1 reactivation in mice, as data have shown that sympathomimetic amines, such as with epinephrine iontophoresis, modulate HSV-1 reactivation [44]. Propranolol suppressed shedding of HSV-1 in murine models after hyperthermic stress [62]. This suggests that further study of the adrenergic pathway in HSV-1 reactivation may illuminate cellular events involved in viral reactivation [62].

4.4. Recurrent Eye Disease in Mice. Mouse models have not been ideal for studying HSV-1 recurrent disease, because their small corneas are very difficult to assess. Studies of recurrent herpetic eye disease in mice are rare; however, there are some data which report HSV-1 recurrent disease in mice. Shimeld et al. [42] used SLE to assess recurrent disease in mice after Cx and Dx. Dendritic ulceration of mouse corneas was seen after induction in 13% (2/16) of latently infected mice [42].

5. Rabbits

5.1. Latency in Rabbits. Rabbits have been used for *in vivo* experiments with HSV-1 latency. Nesburn et al. [63] were among the first to show that HSV-1 remains latent in the TG between attacks of HSV-1 ocular disease in their experiment with NZW rabbits inoculated with McKrae. Rabbits, like mice, have been used to study the LAT gene's role in the establishment of latency (Figure 1). Bloom et al. [64] used rabbits to study the mutagenesis of a cAMP response element (CRE) within the LAT promoter region. NZW rabbits latently infected with a CRE recombinant virus derived from strain 17Syn+ showed reduced reactivation from latency after adrenergic stimulation. Perng et al. [65] also studied the role of the LAT gene in the establishment of latency in rabbits. They inserted the gene for enhanced green fluorescent protein (EGFP) under control of the LAT promoter in a LAT-negative and a LAT-positive virus. Then they examined the TG of the rabbits, and EGFP was detected in more LAT-positive infected neurons than LAT-negative infected neurons. Their results showed that LAT enhances the establishment of latency in the TG [65]. Clement et al. [66] reported active molecular response in the presence of the viral genome by microarray analysis of host gene expression for comparison between naïve and HSV-1 latent rabbit TG, suggesting equilibrium between the ability of the virus to reactivate and host suppression.

5.2. Spontaneous Reactivation in Rabbits. Rabbits can shed HSV-1 DNA in tears spontaneously if latent with an HPR HSV-1 strain. Rabbits latent with HPR also shed infectious virus and HSV-1 DNA in their tears at frequencies and copy numbers similar to humans [1, 2, 8, 17, 38–41]. Humans shed HSV-1 with almost 100% frequency over a 30-day observation period if latent with HSV-1 [38, 39]. This common feature with humans makes the rabbit eye model a good candidate for HSV-1 research. Table 5 shows data from experiments that have shown up to 95% detection of spontaneous shedding in rabbits latently infected with HPR HSV-1 strains.

Laibson and Kibrick [67] in 1969 reported spontaneous HSV-1 shedding in latently infected rabbits. They performed periodic examinations of NZW rabbits latently infected with the Rodanus strain of HSV-1, assessing for infectious virus and herpetic lesions. Over a three-year period after primary infection, they recorded 112 episodes of spontaneous viral reactivation in 50 rabbits. Corneal changes were observed 28% (31/112) of the time that virus was recovered [67]. Nesburn et al. [68] also showed that NZW rabbits latently infected with McKrae spontaneously shed; 65% (13/20) of eyes spontaneously shed HSV-1 DNA in their tears, and 50% (10/20) had lesions as seen by SLE. Kwon et al. [9] found that shedding of infectious virus (not stimulated) in control rabbits latently infected with McKrae was between 5% and 10%.

5.3. Induced Reactivation in HSV-1 Latent Rabbits. Although latently infected rabbits are known to shed HSV-1 spontaneously, stimuli given to rabbits latently infected with 17Syn+ or McKrae facilitates shedding more frequently and for longer durations (days) [1, 2, 69–72]. Experiments have shown that rabbits shed 100% of the time within 7 days post first stimuli [1, 2].

Rabbits have been used to study induction methods for HSV-1 reactivation from latency, much like mice. Table 6 shows examples of experiments that have induced reactivation in rabbits latently infected with HSV-1. Rabbits have been used to elucidate a model for adrenergic viral reactivation [9, 72–76]. Some of the first experiments showing induced reactivation of HSV-1 in rabbits used electrical and mechanical stimulation of the TG [77, 78]. Nesburn et al. [77] manually stimulated the TG of NZW rabbits latently infected with McKrae and reported that the direct trauma to the TG produced ocular HSV-1 shedding in 83% (10/12) of eyes [77]. Green et al. [79] used electrical current delivered to the TG to cause multiple episodes of reactivation in a single rabbit, a model well suited for antiviral testing.

Reports have shown that epinephrine iontophoresis induces HSV-1 shedding in latently infected rabbits reliably and with a high frequency (see Table 6). Hill et al. [72] examined the mechanism of epinephrine induction of HSV-1 reactivation by studying the effects of levo- and dextroepinephrine iontophoresis in rabbits. One hundred percent (5/5) of rabbits responded when administered higher doses of epinephrine. When rabbits were administered lower doses, D-epinephrine-induced ocular shedding was significantly less than L-epinephrine-induced shedding. From this rabbit model, the authors concluded that induction of HSV-1 ocular shedding by epinephrine is a stereoselective, receptor-mediated event that involves sympathetically innervated structures [72]. Shimomura et al. [71] also studied the mechanism of adrenergically induced HSV-1 reactivation from latency in rabbits. Iontophoresis of 6-HD, which causes degeneration of sympathetic nerve terminals in the anterior segment of the eye, enhanced the pharmacologic action of topical epinephrine for inducing ocular HSV-1 shedding [71]. 6-HD produces supersensitivity to adrenergic agonists; therefore, this experiment in the rabbit model confirms that adrenergic neural elements could act as a trigger for HSV-1 reactivation in latency [71].

5.4. Rabbits as an Ocular Model for Therapeutic Intervention Studies. Experiments with adrenergic inhibitors have also attempted to elucidate an adrenergic mechanism of HSV-1 reactivation from latency. Systemic administration of the adrenergic inhibitor propranolol blocks spontaneous HSV-1 ocular shedding and recurrent corneal disease in rabbits. Kaufman et al. [80] found that in NZW rabbits latently infected with 17Syn+ or McKrae, propranolol reduced the amount of spontaneous HSV-1 shedding without any induced stressor. Paradoxically, Hill et al. [81] reported that timolol, an adrenergic antagonist, induced HSV-1 reactivation in latently infected rabbits super sensitized by 6-HD. Epinephrine, an adrenergic agonist, and timolol, an adrenergic antagonist, have the same effect of decreasing intraocular pressure, and it appears that they also have the same effect on induction of HSV-1 in latently infected rabbits [81]. Garza and Hill [82] studied the effect of propranolol on HSV-1 reactivation from latency (Table 7). The authors found that following either epinephrine iontophoresis or immunosuppression, systemic propranolol administration had no effect on HSV-1 reactivation from latency as compared to systemic saline administration [82]. Because propranolol did not block HSV-1 induction in this rabbit model, researchers concluded that HSV-1 induction of reactivation in the rabbit may have a separate pathway from spontaneous reactivation [82]. Since rabbits spontaneously shed frequently when latent with a HPR HSV-1 strain, elucidating this mechanism could enhance the rabbit model of HSV-1 reactivation from latency.

Rabbit eye models have been used to study the effects of corneal manipulation of HSV-1 reactivation from latency (Table 8). Procedures that damage the cornea, such as radial keratotomy (RK) and PKP, induce reactivation of HSV-1 in humans [1, 2, 10]. NZW rabbits latently infected with HSV-1 McKrae strain had a statistically significant increase in the amount of infectious HSV-1 shedding and recurrent corneal lesions following RK [10]. The increased incidence of post-PKP graft failure in those with HSV-1 keratitis was studied in rabbits by Beyer et al. [83]. NZW rabbits were infected with McKrae strain and underwent autograft PKP with or without Dx. In operated eyes without Dx, positive cultures were detected in 20% (2/10) of the eyes; 82% (9/11) had positive cultures after PKP with Dx. There was a significantly greater incidence of superficial punctate keratitis (SPK) in eyes after PKP compared to unoperated eyes latently infected with HSV-1, and Dx significantly increased the incidence of SPK in operated eyes. Eyes treated with PKP and Dx had a significantly increased incidence of stromal keratitis compared with unoperated eyes and eyes after PKP without Dx treatment. The authors concluded that PKP and Dx induce HSV-1 reactivation and recurrent disease in latently infected rabbits, providing a useful tool for studying the development and treatment of recurrent HSV-1 after PKP [83]. Beyer et al. [84] showed that manipulation of corneal nerves induces HSV-1 reactivation in latently infected rabbits. They used cryogenic

Table 6: induction of HSV-1 reactivation in latently infected rabbits.

HSV-1 strain	Type or method of induction	Frequency of detection of reactivation	Use of model	Authors, year (reference no.)
McKrae	Epinephrine iontophoresis	21/28 (75%) of eyes	Experimental induction	Kwon et al., 1981 [9]
McKrae	Epinephrine iontophoresis	9/10 (90%)	Experimental induction	Kwon et al., 1982 [73]
McKrae	Epinephrine iontophoresis	15/20 (75%)	Experimental induction	Hill et al., 1983 [74]
Rodanus	Intramuscular epinephrine	4/6	Experimental induction	Laibson and Kibrick, 1966 [75]
Rodanus	Intramuscular epinephrine	12/22	Experimental induction	Laibson and Kibrick, 1967 [76]
McKrae	Dextro- versus levoepinephrine iontophoresis	5/5 (100%) with 0.01% both levo and dextro with 0.005% epi, 5/10 (50%) with dextro, 10/10 (100%) with levo	Experimental induction	Hill et al., 1985 [72]
McKrae	Dextro- versus levoepinephrine iontophoresis		Experimental induction	Hill et al., 1985 [72]
McKrae	Iontophoresis of 6-HD with topical epinephrine	17/17 (100%) with both, 6/10 (60%) with epinephrine; 6/12 (50%) with 6-HD	Experimental induction	Shimomura et al., 1983 [71]
McKrae	Timolol 0.01% iontophoresis	18/18 (100%)	Experimental induction	Hill et al., 1987 [81]
McKrae	6-HD iontophoresis followed by topical 5% timolol	12/12 (100%)	Experimental induction	Hill et al., 1987 [81]
McKrae	Mechanical stimulation of TG	10/12 (83%)	Experimental induction	Nesburn et al., 1977 [77] 1976 [78]
McKrae	Electrical induction of TG	19/23 (83%) of stimuli	Experimental induction	Green et al., 1981 [79]
McKrae	RK reactivation	15/140 (11%)	Reproduce induction in animal model	Haruta et al., 1987 [10]
McKrae	PKP	2/10 (20%)	PKP induction of reactivation	Beyer et al., 1989 [83]
McKrae	PKP and immunosuppression	9/11 (82%)	PKP induction of reactivation	Beyer et al., 1989 [83]
17Syn+	BN52021	9/10 (90%)	Cryogenic induction with lipoxygenase pathway inhibitor	Beyer et al., 1989 [83]
McKrae	Cx and Dx	21/24 (88%)	Induction	Haruta et al., 1989 [88]
McKrae	Cryogenic injury	5/7 (71%)	Induction by corneal nerve disruption	Beyer et al., 1990 [84]
McKrae	Anterior superficial keratectomy	8/12 (67%)	Induction by corneal nerve disruption	Beyer et al., 1990 [84]
McKrae	Transsection at corneoscleral limbus	8/12 (67%)	Induction by corneal nerve disruption	Beyer et al., 1990 [84]
W Strain	Xalatan	18/24 (75%)	Induction	Gordon et al., 2003 [15]
McKrae	Nicotine transdermal	20/20 (100%)	Induction	Myles et al., 2003 [86]
McKrae	Antinerve growth factor antibody	15/16 (93%)	Experimental induction	Hill et al., 1997 [87]
McKrae	Bupropion	20/20 (100%)	Inhibition of nicotine reactivation	Myles et al., 2004 [89]

Cx: cyclophosphamide, Dx: dexamethasone, PKP: penetrating keratoplasty, and RK: radial keratotomy.

Table 7: Inhibitor of HSV-1 reactivation in latently infected rabbits.

HSV-1 Strain	Inhibitor	Frequency of detection of reactivation with inhibitor	Frequency of detection of reactivation without inhibitor	Authors, year (reference no.)
McKrae	Propranolol with epinephrine iontophoresis	56/84 (67%) with 20 mg/kg; 23/42 (55%) with 200 mg/kb	46/84 (55%) and 20/42 (48%)	Garza and Hill, 1997 [82]
McKrae	Propranolol with Cx and Dx	27/56 (48%)	29/63 (46%)	Garza and Hill, 1997 [82]
17 Syn+	Propranolol with epinephrine iontophoresis	43/70 (61%) with 5 mg/kg;29/56 (52%) with 200 mg/kg	34/70 (49%) and 7/8 (88%)	Garza and Hill, 1997 [82]
17 Syn+	Propranolol with Cx and Dx	24/63 (38%)	21/56 (38%)	Garza and Hill, 1997 [82]
McKrae	Propranolol	20/264 (7.6%)	28/216 (13%)	Kaufman et al., 1996 [80]
17 Syn+	Propranolol	11/336 (3.3%)	31/264 (11.7%)	Kaufman et al., 1996 [80]
McKrae	Bupropion	9/20 (45%)	13/29 (65%)	Myles et al., 2004 [89]
McKrae	Bupropion with nicotine transdermal	13/36 (36.1%)	35/36 (97.2%)	Myles et al., 2004 [89]
W strain	Alpha blockers	10/14 (71%)	13/14 (93%)	Gordon et al., 1990 [90]

Cx: cyclophosphamide and Dx: dexamethasone.

Table 8: Induced recurrent corneal lesions in HSV-1 latently infected rabbits.

HSV-1 strain	Frequency of detection of reactivation	Type or method of induction	Authors/year (reference no.)
McKrae	9/10 (90%) SPK; 1/10 (10%) dendrites; 9 epithelial ulcers	PKP	Beyer et al., 1989 [83]
McKrae	11/11 (100%) SPK; 1/11 (9%) dendrites; 9/11 (82%) epithelial ulcers	PKP plus Dx (immunosuppressant)	Beyer et al., 1989 [83]
17Syn+	5/10 (50%); 2/10 (20%) of controls	BN 52021	Beyer et al., 1989 [85]
McKrae	36/157 (23%)	0.1% 6-HD iontophoresis followed by topical 0.1% Propine	Hill et al., 1987 [116]
McKrae	20/24 (83%)	Cx and Dx	Haruta et al., 1989 [88]
McKrae	17/34 (50%)	0.01% timolol iontophoresis	Haruta et al., 1987 [10]
McKrae	7/12 (58%)	transsection at corneoscleral limbus (corneal nerve disruption)	Beyer et al., 1990 [84]

Rabbits were inoculated in the eye. Lesions were identified using slit-lamp examination. Cx: cyclophosphamide, Dx: dexamethasone, PKP: penetrating keratoplasty, and SPK: superficial punctate keratitis.

injury, anterior superficial keratectomy, and transsection of the corneal nerves at the corneoscleral limbus to induce corneal injury. Significantly more HSV-1 infectious virus was shed after corneal disruption than in controls. Corneal nerve damage induced HSV-1 reactivation from latency [85].

Several experiments have used compounds that enhance the stress response to induce HSV-1 shedding in latently infected rabbits (see Table 7). Myles et al. [86] used nicotine, a compound known to be involved in stress-associated immunomodulation, to induce HSV-1 reactivation in rabbits latently infected with HSV-1 strain McKrae. Transdermal nicotine application to latently infected rabbits yielded 100% (20/20 eyes) ocular shedding as compared with controls in which 70% (14/20 eyes) had spontaneous shedding. The authors hypothesized that nicotine induces HSV-1 reactivation in the rabbit eye model because of its ability to modulate peripheral endocrine and central neuroendocrine receptors in the animal [86]. Nicotine induces HSV-1 reactivation most likely by initiating the stress response, releasing epinephrine and norepinephrine from the adrenal medulla. Hill et al. [87] also found a novel approach to inducing the stress response using antinerve growth factor (NGF) antibody to induce HSV-1 reactivation from latently infected rabbits. NGF, required for survival and differentiation of sympathetic and sensory neurons, is involved in the physiological homeostasis of adult differentiated neurons. It is thought to share a common second messenger pathway with heat or cold stress-induced reactivation of latent HSV-1. In this experiment, researchers found that rabbits treated with anti-NGF antibody had HSV-1 shedding in 93% (15/16) of eyes, as compared to controls with 38% (6/16) of eyes spontaneously shedding [87].

Lipoxygenase products and inflammatory pathway inhibitors have been studied as factors involved in HSV-1 reactivation in latently infected rabbits. Beyer et al. [85] administered BN52021, a platelet-activating factor antagonist, to latently infected rabbits and analyzed reactivation after cryogenic-induced lesions. Rabbits given the antagonist had significantly more positive cultures than controls, and geographic ulcers persisted for a longer amount of time in the affected group than in controls. This method of induction is similar to that of steroids and

NSAIDs in its inhibition of arachidonic acid metabolism [85]. Haruta et al. [88] showed that immunosuppression with Cx and Dx induced HSV-1 shedding and produced recurrent corneal epithelial lesions characteristic of HSV-1 in rabbits, without compromising the structural integrity of the corneal epithelium. Therefore, Cx and Dx are ideal immunosuppressants for experiments investigating recurrent HSV-1 corneal lesions.

In glaucoma patients treated with Xalatan (0.005% latanoprost, Pharmacia Corp., Peapack, NJ, USA), there is an increased incidence of recurrent herpetic ulcers [15]. Gordon et al. [15] used this concept in rabbits to study the effects of Xalatan on spontaneous shedding and induction from rabbits latently infected with HSV-1. Xalatan or its separate individual components were administered to latently infected rabbits, and intrastromal injection of sterile deionized water was used for induction. The results of the experiment showed that neither Xalatan nor its individual components caused an alteration in induced HSV-1 shedding in latently infected rabbits when compared with untreated controls. There were also no significant differences between Xalatan-treated and control groups when rabbits were evaluated for spontaneous shedding.

5.5. Inhibition of Induction in Rabbits. Rabbits have been used to study methods of preventing HSV-1 reactivation from latent infection. Table 7 reports examples of methods that have shown decreased reactivation after induction from HSV-1 in latently infected rabbits. There is anecdotal evidence suggesting that patients taking bupropion (Zyban-SR, GlaxoSmithKline, Greenville, NC, USA), an atypical antidepressant used for smoking cessation, have a decreased incidence of HSV-1 recurrent disease. The mechanism of action of bupropion involves inhibition of norepinephrine, serotonin, and dopamine neuronal uptake. This decreased level of neurotransmitters, especially norepinephrine, is thought to account for the decrease in spontaneous recurrence of HSV-1 lesions in patients using bupropion. Myles et al. [89] used NZW rabbits latently infected with McKrae to show that bupropion inhibits nicotine-induced shedding of ocular HSV-1 *in vivo*, and bupropion alone inhibits HSV-1 shedding compared to controls.

Kaufman et al. [80] reported propranolol suppression of HSV-1 reactivation from latently infected rabbits. Their results showed reduced reactivation of rabbits latently infected with HSV-1 as evidenced by the decreased amount of time that herpetic lesions were present, the decreased frequency of viral shedding, and the decreased number of episodes per eye in rabbits treated with propranolol as compared with controls (see Table 7).

Gordon et al. [90] reported the effect of alpha blockers, thymoxamine, and corynanthine, on HSV-1 reactivation in latently infected rabbits and mice. Their results contributed to the hypothesis that host differences play a key role in the process of HSV-1 reactivation from latency. When comparing the effects of alpha blockade on HSV-1 W strain reactivation from latency after epinephrine iontophoresis, the researchers found significantly less reactivation in treated BALB/c mice, but no statistically significant difference in reactivation of treated NZW rabbits from controls [90]. This experiment suggests a complex difference in the pharmacologically mediated HSV-1 reactivation process between mice and rabbits. The exact mechanism to explain this difference has yet to be discovered.

5.6. Recurrent HSV-Specific Corneal Lesions in Rabbits. While mice are useful for the study of HSV-1 induction and reactivation from latency, they are not as appropriate an animal model for studying patterns of recurrent HSV-1 specific ocular lesions and complications. Rabbits, however, have been an extremely useful animal model for studying HSV-1 recurrent ocular lesions. Several methods have been used to induce herpetic corneal lesions in rabbits [80, 83–85, 88]. Recurrent herpetic eye disease in rabbits is much easier to assess than in mice, because they are better suited for SLE. Table 8 shows data from experiments which recorded increased herpetic corneal lesions when rabbits latent with HSV-1 were induced.

Induction of HSV-1 corneal lesions has been accomplished by both adrenergic iontophoresis and immunosuppression, which both yield deep punctuate, dendritic, and geographic corneal epithelial lesions [91]. For example, Haruta et al. [88] documented corneal lesions in eyes of NZW rabbits latently infected with McKrae after induction with Cx and Dx. The ratio of positive days of epithelial lesions per total days was 44% (82/187). The lesions were correlated with the number of positive swabs recorded during the experiment, confirming that Cx and Dx induce HSV-1 shedding and corneal epithelial lesions in rabbits latently infected with McKrae [88]. Beyer et al. [83] found that PKP induces the recurrence of epithelial lesions in NZW rabbits latently infected with McKrae. Kaufman et al. [80] reported that propranolol suppressed the frequency HSV-1 corneal lesion recurrence after hyperthermic stress of rabbits latently infected with 17Syn+ or McKrae as compared to controls (Table 9).

6. Statistical Analysis of Ocular HSV-1 Data

This section will examine and explain some methods of statistical analyses that deal with quantification of viral particles or viral DNA, where viral DNA copy numbers are taken as surrogate variables for viral copy numbers. The importance of gene expression data, immunological function, and so forth, is great. In this section, we focus on statistical methods appropriate for quantification of viral particles and HSV-1 copy numbers as experimental outcomes in many of the reviewed animal models in HSV-1 eye disease.

Statistical methods in the biomedical literature, when surveyed by statisticians, are often found to be inadequate. Such shortcomings of statistical technique have also been noted in the literature of virology [92]. More uniform statistical methods applied in animal models of HSV-1 eye disease will facilitate meta-analytic studies attempting summarization of results from many studies conducted over

Table 9: Reagents or methods that can inhibit or induce HSV-1 reactivation for rabbits and mice.

Reagents or methods	Mice latently infected with HSV-1	Rabbits latently infected with HSV-1	Reference(s)
Epinephrine iontophoresis	Induces [44]	Induces [9, 72–74, 90]	Willey et al., 1984 [44] Gordon et al., 1990 [90] Kwon et al., 1981 [9] Kwon et al., 1982 [73] Hill et al., 1983 [74] Hill et al., 1985 [72]
UV-B	Induces [42, 114]		Shimeld et al., 1990 [42] Kriesel et al., 1997 [114]
Cx and Dx	Induces [49, 58]	Induces [88]	Cook et al., 1991 [49], Higaki et al., 2009 [58], Haruta et al., 1989 [10] Haruta et al., 1989 [88]
NaB in PBS	Induces [51]		Neumann et al., 2007 [51]
Hyperthermia	Induces [56, 58, 61, 62, 114, 115]		Gebhardt and Kaufman, 1995 [62] Kriesel et al., 1997 [114] Gebhardt et al., 2004 [61] Gebhardt et al., 2004 [56] Gebhardt et al., 2005 [115] Higaki et al., 2009 [58]
Propranolol	Inhibits [62]	Inhibits [80]	Gebhardt and Kaufman, 1995 [62] Kaufman et al., 1996 [80]
Anti-IL-6 antibodies	Inhibits [114]		Kriesel et al., 1997 [114]
ASA	Inhibits [115]		Gebhardt et al., 2004 [56]
Celecoxib	Inhibits [115]		Gebhardt et al., 2005 [115]
Bromfenac	Inhibits [58]		Higaki et al., 2009 [58]
Alpha blockers	Inhibits [90]		Gordon et al., 1990 [90]
6-HD iontophoresis		Induces [81]	Hill et al., 1987 [81]
Timolol		Induces [81, 117]	Hill et al., 1987 [81] Haruta et al., 1988 [117]
Mechanical stimulation of TG		Induces [77]	Nesburn et al., 1977 [77]
Electrical stimulation of TG		Induces [79]	Green et al., 1981 [79]
PKP		Induces [83]	Beyer et al., 1989 [83]
Platelet activating factor antagonist		Induces [85]	Beyer et al., 1989 [85]
Cryogenic injury		Induces [84]	Beyer et al., 1990 [84]
Anterior superficial keratectomy		Induces [84]	Beyer et al., 1990 [84]
Transsection at corneoscleral limbus		Induces [84]	Beyer et al., 1990 [84]
Xalatan		Induces [15]	Gordon et al., 2003 [15]
Nicotine transdermal		Induces [86]	Myles et al., 2003 [86]
Antinerve growth factor antibody		Induces [87]	Hill et al., 1997 [87]
Bupropion		Inhibits [89]	Myles et al., 2004 [89]

years of research, funding, and publication. Eventually, such uniformity of practice will result in greater value of the combined efforts of many research groups over years of effort and, perhaps, even using different animal models of HSV-1 eye disease. Thus, while our comments on statistical methods in HSV-1 disease models will of necessity be a brief section of a review, our hope is this will provide a guide that will lead investigators to consider these fundamental aspects of statistical design and analysis more closely and perhaps to seek statistical consultation on these often difficult methodological problems prior to experimental design or statistical analysis.

Perhaps most important among the statistical issues in analysis of viral counts (obtained by whatever means) is that analysis of frequencies or count data must be addressed using a body of statistical methods known as categorical models,

or methods for the analysis of frequencies [93]. In some cases, count data may contain large count values and may be analyzed as if the count variable was a continuous variable. In contrast, relatively small sample sizes and sparse data which could include zeros, as well as truncated distributions often characteristic of count data, make this naïve assumption—that discrete variables act like continuous variables—a potential cause of problems for statistical estimation and hypothesis testing. Variables used as outcomes in mouse and rabbit models of HSV-1 eye disease may also represent ordinal or nominal scores as well as count data. One such example is an investigator-tallied score for corneal involvement with HSV-1 lesions or a scoring of the severity of associated pathological characteristics such as amount of pus or inflammation. Different statistical methods are appropriate for these different outcome variable types.

Often an additional level of complexity in HSV-1 animal models is observation over time. Experimenters may be concerned with the detailed dynamics of a disease process over time in addition to treatment averages at the end of a period of observation. This adds complexities related to correlations found among observations coming from one experimental subject over time (called within subject correlations). If these correlations within subjects are not dealt with correctly in the experimental design and analysis, they can inflate the test statistics and distort the conclusions of the statistical analysis. The dynamics of the infectious process may be the most important aspect of the HSV-1 animal model, and changes over time must be properly modeled and evaluated statistically. Time series or so-called "repeated measures models" represent an additional level of complexity where outcomes are frequencies.

One assumption which underlies the correct application of parametric statistical methods such as the analysis of variance (ANOVA) is an assumption concerning the normal distribution. This assumption is frequently a source of confusion for nonstatistically trained consumers of statistical analyses. It is not the distribution of the values of the variables under analysis that this assumption addresses; thus the frequently mentioned (erroneous) idea that if values of a variable are not normally distributed, parametric methods cannot be applied, and discrete or nonparametric methods must be used. It is rather the distribution of the means of samples of a variable that we expect to be normally distributed, and this is the assumption our application of parametric methods requires. This approach to the normal distribution of the means of samples from a random variable is addressed in statistical theory by the central limit theorem [94]. This theorem provides that the means of samples from any distribution (normal or not) will approach the normal distribution in the long run with repeated sampling. Parametric methods such as the t-test or ANOVA will perform in a reliable manner if the assumption of normality is violated or not. These tests exhibit a property of statistical methods called robustness.

The assumption of the equality of the variance of each sampled population causes more substantial problems with the performance of statistical estimators. Violation of this assumption causes us to turn to categorical or nonparametric methods rather than the normal distribution of sample means. Means from samples which include varying numbers of high counts or zeros are likely to be unequal in variance. This situation is typical in counting herpetic lesions or in counts of viral particles and is characteristic of processes that are Poisson distributed or distributed in accord with other random discrete distributions.

Simple chi-square tests of a basic kind taught in statistics 101 courses are useful when viral counts are compared between treatments without time observations. A more conservative alternative is the use of exact versions of contingency table tests. These originated in the Fisher's exact test, which was originally applied to 2×2 contingency tables [95], but have been extended to $n \times n$ contingency tables in the modern era of cheap computational power [96]. Power analyses for such experiments and considerations of subdividing the effects which account for overall significance have been described in basic nontechnical terms [97, 98].

Perhaps more useful but more challenging are analytic methods applying generalized linear models [99]. Here the analyst has much greater flexibility to model multiple simultaneous experimental effects such as treatments and their effects over time, as well as to control for "nuisance variables" such as groups or litters of animals. Many different "link" functions can be applied in such models, providing linear additive models type treatment of essentially nonlinear response functions. Extensions of generalized linear models have been developed and are widely applied to observations of subjects over multiple time periods, such as are those often obtained in animal experiments where individual animals are assessed at multiple time points [100].

Many responses are nonlinear, such as the responses that are often seen in pharmacological experiments, or survival data where observations may be incomplete at the conclusion of the experiment (censored observations). In these cases, modern methods of analysis that specifically deal with nonlinear responses allow analysts flexibility to deal with random and fixed effects in the same models of animal testing [101]. More ability to deal with the complexities of experimental design increases the demands for the expertise of the analyst, as more parameters must be assessed and determined and more diagnostics conducted to assess model fitness. As is said in statistical consulting, "what must be taught to clients is not how to do statistics, but when to ask for a statistical consultant."

7. Summary

This paper provides information and examples of animal models of ocular HSV-1 latency, reactivation, and recurrent disease. Both rabbit and mouse eye models have been successful for studying ocular HSV-1, and each model resulted in new information and discoveries related to human HSV-1 ocular disease. The rabbit eye model has been especially important for investigating viral reactivation and recurrent ocular disease. The mouse model has been useful for studying latency, reactivation, and genetics involved with ocular HSV-1.

Though HSV-1 latency, reactivation, and recurrent disease are not fully understood and the mechanisms are still unknown, therapy for HSV-1 recurrent disease has improved significantly since the first antiherpetic was used successfully in 1962. Continued improvement will likely occur through combination therapy as investigators continue to do research. Also, a better understanding of host and viral genetics should allow improved clinical management of recurrent ocular HSV-1, especially herpetic stromal diseases. Mice and rabbit eye models will continue to play a critical role in this important vision research.

Abbreviations

ACV:	Acyclovir
ApoE:	Apolipoprotein E
ASA:	Aspirin
Cx:	Cyclophosphamide
Dx:	Dexamethasone
HPR:	High phenotypic reactivator
HSK:	Herpes stromal keratitis
HSV-1:	Herpes simplex virus type 1
IL-6:	Interleukin 6
INF:	Interferon
LAT:	Latency-associated transcripts
LPR:	Low phenotypic reactivator
NGF:	Nerve growth factor
NZW:	New Zealand White
PKP:	Penetrating keratoplasty
PTG:	Pterygopalatine ganglion
RK:	Radial keratotomy
SCG:	Superior cervical ganglion
SLE:	Slit-lamp exam
SPK:	Superficial punctate keratitis
TG:	Trigeminal ganglion
UVA, UVB:	Ultraviolet.

Conflict of Interests

None of the authors has a proprietary interest in anything mentioned in this article.

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