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**PERSISTENT INFECTION WITH BOVINE HERPESVIRUS-1: A RABBIT
MODEL**

Iowa State University

PH.D. 1981

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Persistent infection with bovine
herpesvirus-1: A rabbit model

by

Daniel Leo Rock

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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Iowa State University

Ames, Iowa

1981

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	5
PART I. PERSISTENT INFECTION WITH BOVINE HERPESVIRUS-1: A RABBIT MODEL	30
ABSTRACT	32
THE RABBIT MODEL	33
REFERENCES	43
PART II. THE PATHOGENESIS OF ACUTE AND RECURRENT BOVINE HERPESVIRUS-1 (INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS) INFECTION IN RABBITS FOLLOWING CONJUNCTIVAL INOCULATION	45
SUMMARY	47
INTRODUCTION	48
MATERIALS AND METHODS	49
RESULTS	53
DISCUSSION	77
ACKNOWLEDGMENTS	81
REFERENCES	82
SUMMARY AND CONCLUSIONS	84
REFERENCES	87
ACKNOWLEDGMENTS	99

INTRODUCTION

Bovine herpesvirus-1 (BHV-1) is responsible for a variety of disease conditions in cattle. Included among these are rhinotracheitis, conjunctivitis, vulvovaginitis, balanoposthitis, meningoencephalitis, and fatal systemic infection (45,70).

A significant aspect of infection with BHV-1 is the ability of the virus to persist beyond the acute infection in inoculated animals. As early as 1910, epidemiological evidence suggested that BHV-1 might persist in infected cattle (107). Since then a large body of literature has confirmed this early suggestion and provided a basic, although not complete, understanding of persistent BHV-1 infection.

Bovine herpesvirus-1 establishes a persistent infection in most, if not all, experimentally infected cattle. Sheffy and Davies (105) found that 100% of 18 previously infected animals were persistently infected. Straub (114) and Davies and Duncan (22) also found 100% rates of persistent infection in previously infected cattle.

Intermittent shed of BHV-1 is characteristically seen in persistently infected cattle. On two occasions, McKercher et al. (65) isolated BHV-1 from nasal secretions of cattle 2-3 months after experimental inoculation. Snowdon (107) found that BHV-1 could be recovered intermittently from infected cattle over a period of 578 days. Multiple incidents of spontaneous virus shed also were observed in two BHV-1 infected bulls over a period of approximately two years (9). Recurrent virus shed and

disease have been induced in persistently infected cattle following various experimental treatments. Sheffy and Davies (105) and Davies and Duncan (22) used dexamethasone, a synthetic corticosteroid, to induce recurrent virus shed and disease in persistently infected cattle. Davies and Carmichael (21) found that adrenocorticotrophic hormone and trigeminal neurotomy were both sufficient treatments for the activation and shed of BHV-1 from persistently infected cattle. Superinfection with para-influenza-3 virus also has been shown capable of inducing recurrent BHV-1 infection in persistently infected calves (67).

Like other members of the alpha herpesvirus group, BHV-1 is thought to persist in sensory and autonomic nerve ganglia, and to travel centrifugally via nerves to produce recurrent virus shed and disease (40,75, 78,79). Bovine herpesvirus-1 specific fluorescence was detected in the trigeminal ganglia of dexamethasone treated persistently infected calves prior to the appearance of virus in the nasal mucosa (78). Bovine herpesvirus-1 also has been isolated from the trigeminal ganglia of clinically normal cattle at slaughter (40), although prior attempts by other researchers to isolate virus from ganglia of non-dexamethasone treated experimentally infected cattle were unsuccessful (21,22). Recently, Homan and Easterday (41) have demonstrated the presence of BHV-1 DNA in neurons of the trigeminal and superior cervical ganglia of persistently infected calves.

Many unanswered questions regarding BHV-1 persistence remain. Little is known about the virus-cell-host interaction that is responsible for initiation, maintenance, and reactivation of persistent infection.

In addition, it is not known if the natural course of persistent BHV-1 infection can be modulated. More specifically, can immunologic or physiologic manipulations prevent the establishment of persistent infections in non-infected animals, or prevent the reactivation and shed of virus in animals already persistently infected? The development of a laboratory model of persistent BHV-1 infection may facilitate the study of these and other questions pertaining to persistent BHV-1 infection.

Rabbits have been infected experimentally with BHV-1 and suggested as possible laboratory models for studying the pathogenesis of BHV-1 infection (46,59,60). Lupton et al. (60) have isolated BHV-1 from the trigeminal and optic nerves of rabbits during acute conjunctival infection and have suggested that the potential exists for the establishment of persistent infection.

The objectives of this research were to determine if persistent infections could be established in BHV-1 infected rabbits and to evaluate the rabbit as a laboratory model of persistent BHV-1 infection.

Explanation of Dissertation Format

This dissertation consists of an introduction, a literature review, two separate manuscripts, a general conclusion, references and acknowledgements. The Ph.D. candidate, Daniel Leo Rock is the senior author and principal investigator for each of the manuscripts.

LITERATURE REVIEW

Persistent Bovine Herpesvirus-1 Infection

A characteristic of all herpesviruses is the ability to establish persistent infection (109). It has been shown that BHV-1 establishes a persistent infection in virtually all cattle following inoculation by the vaginal (22,42,107), preputial (9), intravenous (107), or intranasal route (22,105). Persistent BHV-1 infection has been characterized by the following: (1) Persistently infected animals have been reported to shed virus intermittently without evidence of clinical disease (9,42,107), and (2) Virus has been reactivated predictably from persistently infected cattle following various experimental treatments (22,67,105).

Spontaneous reactivation of persistent infection

Bovine herpesvirus-1 has been recovered intermittently from cattle which had been inoculated previously by the vaginal (22,42,107), preputial (9,52,115), intravenous (107), or intranasal route (65). In all of the above reports, recurrent virus shed was detected without the appearance of clinical disease. McKercher et al. (65) isolated virus from nasal washings of animals 2-3 months post-infection. Studdert et al. (115) detected virus in preputial washings of a bull 26 days post-infection, although washings taken at 12, 16, and 19 days post-infection failed to yield virus. Kubin (52) also reported the isolation of BHV-1 from preputial washings taken from a clinically normal bull. Snowdon (107) detected

7 separate periods of virus shed over a period of 578 days in an intravenously inoculated animal. In addition, six periods of virus shed were detected in a bull under observation for 1 year. On 17 different occasions, over a period of two years, Bitsch (9) isolated BHV-1 from preputial washings taken from two persistently infected bulls. The titer of virus recovered in the washings ranged from 10^2 - 10^4 TCID₅₀'s per ml of wash and was lower than the amount of virus seen during the acute infection or following prednisolone injections. Huck et al. (42) observed that intermittent virus shed from persistently infected heifers was more frequent during the first 5 months of a 12 month observation period. Incidents of spontaneous virus shed in the heifers ranged from 1 to 6 days.

Intermittent virus shed has been shown to occur at the original site of inoculation. Bovine herpesvirus-1 was recovered from vaginal but not nasal swabs taken from animals initially infected by the vaginal route (35,42,107). Snowdon (107) detected intermittent BHV-1 shed from both the nose and the vagina of an animal that had been infected by the intravenous route.

Fluctuations of serum neutralizing antibody titer in individual persistently infected animals have been reported by several researchers (9,42,107). Attempts to correlate recurrent virus shed with the presence or absence of serum neutralizing antibody have proven unsuccessful (9,42). Snowdon (107) found that rises in titer followed a period of recurrent virus shed. However, Bitsch (9) found little to no rise in serum neutralizing antibody following periods of virus shed.

Induced reactivation of persistent infection

A variety of experimental treatments has been used in an attempt to induce recurrent BHV-1 infection in persistently infected cattle. One such treatment, corticosteroid treatment, has been shown to induce BHV-1 recurrence (2,9,21,22,24,25,33,44,52,78,79,81,88,105,114). Studies on experimentally infected cattle have demonstrated virus shed in virtually all animals following corticosteroid treatment (9,21,22,105,114). Corticosteroid treatment of naturally infected cattle has resulted in recurrence of infection in 60-100% of the animals treated (24,25,52).

A number of observations have been made on cattle following an initial corticosteroid induced viral recurrence. Davies and Duncan (22) reported that clinical signs of infection were first seen 3 to 4 days after initiation of dexamethasone treatment. Recurrent virus shed was first detected 2 to 4 days post-treatment initiation and persisted for 4-7 days. Similar observations have been made by others (9,21,22,25,44,78,79,80,105). An increase in the anti-BHV-1 serum neutralizing antibody titers of persistently infected animals characteristically was seen following corticosteroid induced recurrence of disease (9,21,44,88,89). However, Dennett et al. (25) and Darcel and Dorward (20) found no significant changes in levels of serum antibody following dexamethasone treatment.

Variations of the observations described above were seen in subsequent corticosteroid induced reactivations attempted on the same animal. Davies and Duncan (22) found a variable pattern of virus excretion when persistently infected animals were re-treated with dexamethasone 4 weeks after the initial treatment. Virus was recovered later and shed for a

shorter period of time following the second treatment. A more typical response was seen when animals received their second treatment 3 months after the initial one, although variable patterns of virus excretion still were observed in some animals. Similar results have been obtained by others (88,89,114).

Davies and Carmichael (21) and Davies and Duncan (22) induced BHV-1 recurrence using adrenocorticotrophic hormone (ACTH). The effectiveness of this treatment was due to increased endogenous corticosteroid release. The recurrent infection observed following ACTH treatment was similar to that seen following corticosteroid treatment.

Trigeminal neurotomy has been suggested as a sufficient condition for reactivation of BHV-1 (21). Virus was recovered from the denervated nostril in 1 of 2 treated calves 11 days post-surgery.

Reactivation of persistently infected calves after experimental infection with parainfluenza-3 virus (PI-3) has been described by Mensik et al. (67). It was suggested that PI-3 virus acted like other non-specific stressing factors in causing recurrent BHV-1 infection.

Snowdon (107) has recovered BHV-1 from a persistently infected cow following each of two sequential calvings. Clinically apparent infection was seen 5 days after the birth of the first calf, but was absent following the second birth.

The immune response and persistent infection

Vaccination of cattle with an inactivated or a modified live BHV-1 vaccine was unable to prevent the establishment of a persistent infection

following a subsequent infection with virulent virus (80,106).

Although vaccination failed to prevent latent infection, a variety of reports suggested a role for the immune response in altering the natural course of recurrent BHV-1 infection. Davies and Carmichael (21) observed that the cellular immune response of dexamethasone treated persistently infected cattle was depressed at the time of virus reactivation. They suggested that the observed defect in cellular immunity was not responsible for viral reactivation, but would account for the apparently unrestricted multiplication of the virus and the development of clinically apparent disease seen in dexamethasone induced recurrent infection. Pastoret et al. (89) have shown enhanced BHV-1 specific lymphocyte blastogenesis, antibody dependent cellular cytotoxicity, and serum neutralizing antibody titers in cattle following dexamethasone induced viral recurrence. The authors have suggested that this enhanced level of immunity was responsible for the reduction in the amount and duration of virus excretion observed in the cattle following a second dexamethasone treatment. Enhanced immunity has also been suggested in explaining the variable virus excretion patterns observed in persistently infected cattle following multiple corticosteroid treatments (22,88).

Some reports have indicated that hyperimmune persistently infected cattle failed to excrete BHV-1 following corticosteroid treatment. Kokles (49) observed that cattle immunized with a BHV-1 live virus vaccine and subsequently infected with virulent field virus could not be reactivated using corticosteroids. In a similar experiment, Pastoret et al. (89)

were unable to induce recurrent BHV-1 shed in cattle that had been vaccinated with a temperature sensitive BHV-1 vaccine and challenged with a virulent virus. In contrast, Straub (114) found that cattle infected with field virus, and subsequently vaccinated, could be reactivated using corticosteroid treatment. In addition, Dennett et al. (25) found no correlation between the initial serum neutralizing antibody titer of naturally infected cattle and the ability to induce BHV-1 recurrence in them. Clarification of this important question will require further study.

The Pathogenesis of Persistent Bovine Herpesvirus-1 Infection

Acute phase of infection

Experimental infection of cattle with bovine herpesvirus-1 by the nasal (4,22,28,34,65,75), conjunctival (77), vaginal (22,76), and preputial route (115) has resulted in localization and replication of virus at the site of inoculation and generalization of the infection to distant, non-inoculated areas. Neural spread, hematogenous spread, lymphatic drainage, and extension of virus infection by multiplication from a primary to a secondary site have been suggested as mechanisms for generalization of BHV-1 infection (4,22,34,65,75,76,85).

Davis and Duncan (22) observed that intranasal infection of cattle with BHV-1 resulted in inflammation of the nasal and conjunctival epithelium, trigeminal ganglionitis, and mild inflammation of the medulla oblongata

and pons. Virus was isolated from the nasal mucosa, conjunctival mucosa, trigeminal nerve, trigeminal ganglia, medulla oblongata, pons, cerebrum, olfactory bulb, adrenal gland, and tissues of the genital tract. Narita et al. (75) using the Los Angeles strain of BHV-1 obtained similar histopathologic results, although more severe lesions were observed in the medulla oblongata and pons. Lesions of the brain stem were located in the main sensory and spinal tract nuclei of the trigeminal nerve, and led the authors to suggest that generalization of infection to the central nervous system (CNS) was accomplished by neural spread.

Further evidence for neural spread of virus to the CNS following intranasal infection was provided by Bagust and Clark (4). Virus was recovered only from the nasal mucosa and tonsils of calves killed 2 days post inoculation, but was present in the mandibular and maxillary branches of the trigeminal nerve and the trigeminal ganglia of calves killed 4 and 5 days post-inoculation. Hall et al. (34) and Edington et al. (28) also have described CNS infection in cattle following intranasal inoculation of BHV-1.

Following conjunctival inoculation of virus, histopathologic lesions were detected in the conjunctiva, trigeminal ganglia, and medulla oblongata. The location of the lesions in the brain stem was consistent with neural spread of infection via the trigeminal and lacrimal nerves (77).

Vaginal infection of cattle with BHV-1, like nasal infection, resulted in wide spread generalization of infection. Davies and Duncan

(22) isolated virus from tissues of the genital tract, sacral spinal cord, and conjunctiva of calves killed 4 days post-infection. In addition, BHV-1 was detected in the sacral and cervical spinal cord, trigeminal ganglia, medulla, pons, cerebrum, spleen, adrenal gland, and nasal mucosa from animals killed 9 and 11 days post-infection. Although virus was isolated from neural tissue, these authors failed to find histopathologic evidence of neural involvement. In contrast, Narita et al. (76) observed early histopathologic lesions in the sacral spinal cord and sacro-lumbar spinal ganglia of vaginally inoculated cows, with later appearing lesions being detected in the trigeminal ganglion, medulla oblongata, and cerebrum. In addition, BHV-1 specific fluorescence was detected in satellite cells of the trigeminal ganglion from a calf killed 12 days post infection. These authors suggested that early and late neural infection resulted from centripetal spread of virus along sensory nerve fibers of the vagina and nasal mucosa respectively. This interpretation was consistent with the virus isolation data of Davies and Duncan (22).

Persistent phase of infection

Attempts have been made to determine what tissues, and more specifically, what cell types harbor BHV-1 between the acute infection and subsequent recurrent infections. McKercher et al. (65), using tissue homogenates for virus isolation, were unable to isolate BHV-1 from respiratory tissues, ocular tissues, the CNS, and the circulatory system

of cattle killed 24 to 290 days post-infection. Bagust and Clark (4) and Edington et al. (28) also were unable to isolate virus from the CNS of calves killed 28 days post-infection. Narita et al. (75) failed to isolate virus or detect virus specific fluorescence in the nasal mucosa, trigeminal ganglia, medulla oblongata, and cerebrum of intranasally infected calves killed 30 to 98 days post-infection. Sheffy and Davies (105) using explant culture for virus isolation, failed to recover BHV-1 from the upper respiratory tract, lower respiratory tract, trigeminal ganglia, medulla oblongata, cerebrum, adrenal gland, and reproductive tract of non-dexamethasone treated persistently infected animals. Similar results using explant culture for virus isolation have been obtained by others (21,22).

Using an explant culture procedure, Homan and Easterday (40) recently have isolated BHV-1 from the trigeminal ganglia of clinically normal cattle. Virus was isolated 7-24 days after explant culture initiation from 10% of the ganglia examined. In addition, these authors have demonstrated the presence of BHV-1 DNA in neurons of the trigeminal and superior cervical ganglia of persistently infected calves (41).

Pathologic changes and BHV-1 specific fluorescence have not been observed in tissues taken from persistently infected cattle (22,75,78,79).

Recurrent phase of infection

The pathogenesis of recurrent BHV-1 infection has been studied following dexamethasone treatment of persistently infected cattle. Davies and Duncan (22) have described recurrent BHV-1 infection in

cattle initially infected by the nasal route. Histopathologic evidence of recurrent infection was confined to the initial site of inoculation and the trigeminal ganglia. However, virus was isolated from the medulla and from vaginal swabs taken during the recurrent infection. Narita et al. (78) observed BHV-1 specific fluorescent neurons in the trigeminal ganglia of calves, initially infected by the nasal route, 3 days after dexamethasone treatment initiation, but were unable to isolate virus from them. The appearance of fluorescent cells in the ganglion 1-2 days before the appearance of virus in the nasal secretions led the authors to suggest that the trigeminal ganglion was the site of virus persistence. Unlike the results of Davies and Duncan (22), Narita et al. (78) found histopathologic evidence of CNS involvement during recurrent BHV-1 infection. Lesions were observed in the medulla oblongata 4 days post-dexamethasone treatment initiation, and were detected in the pons and cerebrum of treated calves 7 days post-treatment initiation. Lesions of the brain stem were confined to the main sensory and spinal tract nuclei of the trigeminal nerve, which suggested virus spread from the ganglia to the brain following dexamethasone treatment.

Recurrent BHV-1 infection in cows initially infected by the vaginal route involved the site of inoculation, the upper respiratory tract, and the nervous system. Davies and Duncan (22) found that histopathologic lesions were confined to the vagina, although virus was isolated from the upper respiratory tract, trigeminal ganglia, medulla, and the cervical and sacral spinal cord. Narita et al. (79) first recovered BHV-1 from vaginal secretions 3 days post-dexamethasone treatment initiation.

Pathologic changes were observed in the lumbosacral spinal cord and the associated sensory ganglia 4 days post-treatment initiation, although virus was not recovered from these tissues. Virus-specific fluorescence was detected in satellite cells of the sacrospinal ganglia 4 days post-treatment initiation, and in Schwann cells of the sensory nerve fibers of the vagina at 6 days post-treatment. The authors concluded that the sacrospinal cord and ganglia were likely sites of BHV-1 persistence in vaginally infected animals.

Persistent Infection with Herpes Simplex Virus

The herpesviruses have been divided into three subfamilies, one of which is the alpha herpesvirus group. This group is made up of herpesviruses that exhibit the following common characteristics: (1) a genome of double stranded DNA from 85-110 x 10⁶ daltons with sequences from both or either terminus present in an inverted form internally; (2) a replicative cycle of less than 24 hours; (3) a variable host range; (4) a virus highly cytopathic in cell culture; and (5) an ability to establish persistent infection in nervous tissue (62).

Persistent infection with herpes simplex virus (HSV) has been extensively studied, and is better understood than the persistent infections established by any other viruses in the alpha herpesvirus group. This understanding is due to the growing amount of information on the molecular biology of the virus itself and the availability of convenient laboratory animal models of persistent infection.

Bovine herpesvirus-1 (BHV-1) is a putative member of the alpha herpesvirus group and shares many common properties with HSV (105). These similarities warrant the inclusion of a review on persistent infection with HSV.

Herpes simplex virus has been shown to be responsible for a variety of disease conditions in man. Included among these are urogenital infections, gingivostomatitis, keratitis, conjunctivitis, cheilitis, meningoencephalitis, and systemic infection (5,72). Persistent infection is thought to occur in most, if not all persons following primary infection with HSV (72). Clinically apparent incidents of recurrent herpetic disease are estimated to occur in 20-40% of the population (108). Asymptomatic recurrent infections also have been described (1,12,96). The pathogenesis of persistent HSV infection in humans, although not directly studied, is inferred from a variety of clinical and experimental observations. Clinical observations initially suggested that the sensory nerves or ganglia were involved in the pathogenesis of persistent HSV infection (5,14,15,47). In addition, virus was isolated from sensory and autonomic nerve ganglia of clinically normal individuals (7,23,31, 58,71,123,124). These two observations, along with extensive corroborative evidence from animal models of persistent HSV infection (19,27,47,86,109), have led to the following generally accepted scheme for the pathogenesis of recurrent HSV infection in humans. Following acute virus infection HSV is transported centripetally via nerves to local nerve ganglia. The virus persists in the ganglia in an as yet unknown state between periods

of recurrent infection. During the persistent phase of the infection virus can not be recovered from ganglionic tissue homogenates, but can be isolated from explant cultures. Following an inducing stimulus, virus is reactivated and transported centrifugally via nerves to the initial site of infection where virus shed with or without the appearance of clinical disease occurs.

Animal models of persistent HSV infection

The mouse model Among the laboratory animal models of persistent HSV infection, the mouse model has been the most extensively studied. The acute infection observed in mice following inoculation with HSV-1 and HSV-2 has been characterized using virus isolation, histopathology, fluorescent and enzyme-linked antibody staining and electron microscopy. Generalization of HSV infection was shown to occur following virus replication at the site of inoculation (16,26,36,50,51,55,97). Neural spread of HSV has been considered to be the primary mechanism for the generalization of infection to the central nervous system. Cook and Stevens (16) detected the sequential appearance of HSV-1 in the sciatic nerve, sarco sciatic spinal ganglia, spinal cord, and brain of mice following foot pad inoculation of virus. Lascano and Berria (55), using an enzyme-linked antibody staining procedure, also have demonstrated this sequence of virus spread in mice inoculated by the footpad route. Kristensson et al. (50,51) showed that HSV infection was detected first in the trigeminal ganglion and later in the brain stem of animals

inoculated via the corneal route. The location of the brain stem lesions suggested that HSV infection of the central nervous system resulted from neural spread of virus. Price and Schmitz (91) observed that post-ganglionic neurectomy carried out before intraocular infection with HSV-1 totally eliminated the spread of virus to the superior cervical ganglion.

Although neural spread of HSV has been generally accepted as the primary means for the spread of virus to the central nervous system, the exact mechanism responsible for neural spread has remained quite controversial. Retrograde axonal transport of virus was suggested as one possible mechanism for neural spread. Kristensson et al. (50) found that horseradish peroxidase, a protein tracer known to be transported by retrograde axonal transport, was transported from the site of viral inoculation to neurons of the sensory ganglion corresponding to those infected with HSV. In addition, Hill et al. (36), Cook and Stevens (16) and Yamamoto et al. (127) observed, using electron microscopy, viral particles in axons near neuronal perikarya, but failed to observe them distal to that area. A variety of indirect observations also were advanced as evidence supporting axonal transport of virus. Cook and Stevens (16) argued that axonal spread would account for: 1) the rapid appearance of virus in the ganglion; 2) the observation that neurons are infected first, with infection then spreading to other ganglion cell types; and 3) the observation that infection progresses even in the presence of high levels of neutralizing antibody.

Others have suggested that neural spread is best explained by cell to cell transmission along nerve fibers or by the passive motion of virions along the intercellular space of the nerve fiber. Lascano and Berria (55) found extensive replication of HSV in Schwann cells of the sciatic nerve, while failing to detect any viral particles within axons. They concluded that cell to cell spread of infection was consistent with their observations. In contrast, Dillard et al. (26) and Cook and Stevens (16) observed that Schwann cells associated with peripheral nerves replicated HSV poorly, if at all.

Factors extrinsic and intrinsic to nerve ganglia have modified the course of acute HSV ganglionic infection in mice. Tenser and Dunstan (116) found that thymidine kinase-deficient mutants of HSV showed impaired replication in ganglionic neurons, even though virus titers observed in ocular tissues were similar to those seen with wild type virus. Price and Schmitz (91) observed that postganglionic neurectomy, performed after virus had reached the superior cervical ganglion, augmented the acute phase of the infection. Intraganglionic viral replication also was prolonged by using athymic nude mice or immunosuppressed conventional mice (87,91).

Persistent HSV infection was established in almost all mice that survived the acute infection (17,61,110,119). Following the acute phase of infection, latent virus was isolated, using explant procedures, from the dorsal root ganglia, autonomic ganglia, spinal cord, brain stem, and adrenal gland of persistently infected mice (17,48,64,92,110,119).

Cook and Stevens (17) have shown, following intravenous inoculation of virus, that 83% and 25% of the mice harbored latent HSV-1 in the dorsal root ganglia and central nervous system respectively. Tobin et al. (119) observed that 80% of the mice harbored latent virus in the pelvic sensory ganglia following cervicovaginal inoculation with HSV-2. Unlike other reports, Hill et al. (39) recently isolated HSV from the skin of clinically normal persistently infected mice, and suggested that the skin also may be a site of viral latency.

Herpes simplex virus DNA has been detected in the trigeminal ganglion and brain of persistently infected mice (13,93,104). Puga et al. (93) found that HSV DNA was present in the trigeminal ganglion at a level of 0.1 genome equivalents per cell during the persistent phase of the infection. Cábera et al. (13) reported that 30% of the brains from persistently infected animals contained HSV DNA sequences, although latent virus was isolated from only 5%.

Spontaneous incidents of recurrent herpetic disease in persistently infected mice were observed by Hill et al. (37). Recurrence of infection was detected in approximately 50% of the persistently infected animals monitored over a 150 day period, and was characterized by erythema of the original virus inoculation site. Virus isolation and electron microscopic procedures demonstrated the presence of HSV in biopsies taken from the lesions.

A variety of experimental treatments has been used on mice in an attempt to induce the reactivation of latent HSV. However, the mechanisms

of action of these reactivation-inducing stimuli are not understood. Stevens et al. (113) demonstrated that severe pneumococcal pneumonia in persistently infected mice lead to reactivation of latent HSV. Virus was recovered from the spinal ganglia and sciatic nerve of treated mice, but recurrent disease was not observed. Price and Schmitz (90) and Waltz et al. (122) found that postganglionic neurectomy induced HSV reactivation in associated ganglia. Preganglionic neurectomy failed to induce viral reactivation in the superior cervical ganglia of persistently infected mice (90).

Trauma to the original site of virus inoculation has proven successful in inducing viral reactivation and recurrent viral disease (10,11,38,43,103). Hill et al. (38) found that mild trauma, which consisted of stripping the originally infected ear with cellophane tape, resulted in recurrent lesions in 30% of the persistently infected mice 2-5 days post-treatment. Herpes simplex virus was isolated from the recurrent lesions. Hurd and Robinson (43) have shown that 47-85% of persistently infected mice developed recurrent lesions following epilation of the hair over the original infection site. Sekizawa et al. (103) observed an increase in the levels of HSV neutralizing antibodies in 90% of the persistently infected mice following dry ice treatment of the original inoculation site. This result was not observed if the animals were treated at a site different from the original inoculation site. The rises in serum neutralizing antibody were considered as evidence of viral reactivation.

Attempts to reactivate latent HSV or induce recurrent disease using immunosuppressive drugs have met with varying degrees of success. Prednisolone treatment, but not hydrocortisone treatment induced the recurrence of disease in 17% of the persistently infected animals 12-30 days post-treatment (212). Openshaw et al. (87) found that cyclophosphamide treatment resulted in the reactivation of latent HSV in the trigeminal ganglia of 70% of the treated animals. In addition, Kurata et al. (53) observed recurrent lip lesions and HSV specific fluorescence in the trigeminal ganglia of persistently infected mice treated with cyclophosphamide. Others have failed to obtain these results following cyclophosphamide treatment of persistently infected mice. Price and Schmitz (90) reported that cyclophosphamide was not effective in reactivating latent HSV in superior cervical ganglia. Blyth et al. (10,11) found that cyclophosphamide treatment failed to increase the incidence of recurrent clinical disease in persistently infected mice.

The rabbit model Rabbits have proven to be an excellent laboratory model for the study of acute and persistent HSV infection. Keratitis (68,69,94), uveitis (69), conjunctivitis (69), and encephalitis (73,74,120) were produced in rabbits following experimental inoculation of HSV-1. Following corneal inoculation, virus or virus-specific fluorescence was detected in the corneal epithelium, the trigeminal ganglia, and the brain stem (6,94,112). Pathologic evidence has suggested that generalization of virus from ocular tissues to the brain occurs by intra-axonal spread of virus (6,120). However, reports have shown that

non-axonal cell to cell spread of virus along nerve fibers also occurs (73,74).

Persistent infection with HSV-1 has been shown to occur in rabbits following corneal inoculation. Nesburn et al. (83) recovered latent HSV, using an explant procedure, from 73% of the trigeminal ganglia examined, but failed to recover virus from the conjunctiva, cornea, iris, nictitating membrane, lacrimal gland, trigeminal nerve, and brain stem of persistently infected rabbits. Stevens et al. (112) also recovered latent virus from 5 of 6 trigeminal ganglia taken from rabbits 4 to 8 months post-inoculation. Similar results have been reported by others (6,94,95). Unlike other reports, Knotts et al. (48) recovered HSV-1 from 5 of 14 brainstems taken from persistently infected rabbits 1-12 months post-inoculation.

Spontaneous reactivation and shed of HSV-1 from persistently infected rabbits have been observed. Nesburn et al. (82) observed spontaneous reactivation in 13 of 20 previously infected rabbits' eyes. Reactivation often was accompanied by clinically evident disease, although reactivations without evidence of disease also were seen. Stevens et al. (112) and Nesburn et al. (83) have reported similar findings with respect to spontaneous reactivation and shed of HSV-1.

Attempts have been made to induce reactivation and shed of HSV-1 from persistently infected rabbits. Laibson and Kibrick (54) found that epinephrine induced reactivation and shed of HSV-1 in some treated animals. Viral reactivation often was accompanied by clinically apparent disease. Nesburn et al. (84) demonstrated that mechanical stimulation

of latently infected trigeminal ganglia resulted in predictable reactivation and shed of HSV-1. Virus was detected in ocular swabs as early as 18 hours post-treatment and was seen in 80% of the animals by 48 hours post-treatment.

The guinea pig model Persistent HSV infections were established in guinea pigs following an acute footpad (30,100), vaginal (3,101), or corneal infection (117). Virus was isolated from the site of inoculation and the nervous system of acutely infected guinea pigs. Scriba (100) isolated HSV-2 from the footpad, sciatic nerve, and the dorsal root ganglia of guinea pigs during acute footpad infection. Following vaginal inoculation of guinea pigs with HSV-2, virus was detected in the lumbosacral dorsal root ganglia, lumbosacral spinal cord, genital nerves, and the sciatic nerve (101). Tenser and Hsiung (117) found HSV-1 in the trigeminal ganglia, brainstem and cerebrum of adult guinea pigs following corneal inoculation of virus.

Spontaneous reactivations of latent HSV with the appearance of recurrent foot pad lesions have been described in persistently infected guinea pigs. Scriba (100) found that 11 of 13 guinea pigs exhibited from 1-7 exacerbations of recurrent disease during a 200 day observation period. Recurrent lesions developed at the site of initial infection and persisted for 2 to 28 days. During periods of recurrent disease virus was isolated from the footpad, sciatic nerve, and the lumbosacral dorsal root ganglia.

In the guinea pig model, latent virus has been recovered, using explant culture, from neural and non-neural tissues. Latent HSV-2 was

isolated from the footpad, sciatic nerve, and the lumbosacral dorsal root ganglia of animals initially infected in the footpad (102). However, latent virus was detected only in the footpad of animals initially infected with HSV-1. Following an initial vaginal infection with HSV-2, latent virus was isolated from the lumbosacral dorsal root ganglia, lumbosacral spinal cord, genital nerves, sciatic nerve, uterus, vagina, footpad, and adrenal gland (101). Tenser and Hsiung (117) observed that the age of the animal at the time of initial HSV-1 infection had an effect on where latent virus was found. Latent HSV-1 was isolated only from the trigeminal ganglia of persistently infected adult guinea pigs killed 21-70 days after corneal inoculation of virus, but was detected in the trigeminal ganglion, trigeminal root, and brain stem of animals that were initially infected as neonates.

Neonatal guinea pigs were found to be more likely to develop persistent HSV-1 infections than adult animals (117). However, treatment of adult guinea pigs with hydrocortisone prior to inoculation of HSV-1 increased the rate of persistent infection in adult animals.

Characterization of Persistent HSV Infection in Nerve Ganglia

Cellular location of persistent virus

A great deal of evidence has suggested that neurons harbor latent HSV. Cook et al. (18) have provided the following evidence in support of the neuron: 1) HSV specific fluorescence was detected first in neurons of reactivating ganglia; 2) virus specific ultrastructural changes were

observed first in neurons of reactivating ganglia and later in surrounding cells; and 3) HSV DNA was detected initially in neurons. In addition, Baringer and Swoveland (6) and McDougall et al. (63) have made indirect observations that are consistent with those of Cook et al. (18). Recently, McLennan and Darby (66), using temperature sensitive (ts) mutants of HSV for animal inoculation and reactivating persistently infected ganglia at the non-permissive temperature, removed the possibility that changes seen in neurons were secondary to a primary reactivation of HSV in another cell type. Viral specific fluorescence was detected in abortively reactivated neurons, but not in any other cell type.

Initiation and maintenance of persistent infection

The evidence available strongly suggests that HSV persists in neurons. The nature of the virus-cell or virus-cell-host interaction ultimately responsible for this phenomenon has not been determined. Two major hypotheses have been advanced to explain HSV persistence, although definitive evidence supporting or refuting either is still lacking (99, 109). The "dynamic state" hypothesis proposes that persistent HSV infections are chronic infections, with virus continually being produced at very low levels in the ganglia. The "static state" hypothesis views the neuron-virus interaction as a stable one. In this model, HSV is harbored within neurons in a non-replicating state. A triggering stimulus is required for viral reactivation and productive replication. The "static state" model or minor variations of it is consistent with experimental observations to date and is generally accepted as a

theoretical base for further experimentation (9,47,98,109).

Viral gene expression is suspected of playing a role in the initiation and/or maintenance of the persistent state, although this has not been directly demonstrated (61). Temperature sensitive mutants of HSV, restrictive at the host body temperature, differed in their ability to establish persistent infection in mice (57). This observation suggested that latency-negative ts mutants had lesions in genes responsible for products crucial to the establishment of latent infection. Gerdes et al. (32) observed that the viral DNA phenotype of HSV ts mutants was not correlated with the ability to establish persistent infection and noted that latency-negative mutants tended to produce more morphologically identifiable viral components than latency-positive mutants. Watson et al. (125) recently demonstrated, using revertants of latency-negative mutants, that one immediate early and one or more later virus gene products were necessary for initiation and/or maintenance of the latent state.

Apart from the studies using ts mutants, attempts have been made to demonstrate viral gene activity in persistently infected ganglia. Puga et al. (93), using a DNA-RNA reassociation kinetics procedure, were unable to detect viral mRNA in nerve ganglia taken from persistently infected mice. McDougall et al. (63) detected viral mRNA in neurons of human thoracic ganglia with an in situ hybridization procedure. However, it is not known if the mRNA-containing neurons represent truly latent cells or cells undergoing spontaneous reactivation.

Reports that thymidine kinase-negative mutants of HSV were less successful at establishing persistent infections than wild type virus

(29,116,118), suggested a possible role for viral thymidine kinase in the initiation and/or the maintenance of the persistent state. Yamamoto et al. (126) detected HSV-1 specific thymidine kinase activity in ganglia of persistently infected mice up until 60 days post-inoculation. These authors concluded that the presence of viral enzymatic activity, weeks after virus was no longer recoverable from tissue homogenates, represented continual or intermittent expression of the viral genome during the persistent phase of the infection. Unlike Yamamoto et al. (126), Fong and Scriba (30) failed to detect any HSV-2 thymidine kinase activity in footpads or lumbosacral ganglia taken from persistently infected guinea pigs.

It is unknown what role the host's immune response plays in the initiation and/or maintenance of persistent HSV infection (56). Stevens and Cook (111) found that anti-viral IgG inhibited intraneuronal viral DNA and antigen synthesis in explanted reactivating ganglia. However, persistent infections have been maintained in mice lacking detectable serum neutralizing antibodies (103).

Reactivation of persistent infection

Spontaneous and induced reactivations of HSV infection have been described in man, mice, rabbits, and guinea pigs (37,83,100,109). Trauma, neurectomy, and immunosuppressive drugs all have induced viral reactivation, with or without the reappearance of clinical disease (15,38,53,87,90,103). Although no direct evidence is available,

hypotheses have been advanced to explain the mechanisms of action of these reactivation-inducing stimuli. Central to the question of induced viral reactivation has been the actual mechanism of viral persistence. Proponents of the "static state" theory of HSV persistence have suggested that reactivating stimuli act directly or indirectly on cells harboring latent virus. Stimuli are thought to induce productive viral replication by altering the existing cell-virus interaction (90, 103). A second hypothesis is favored by those supporting a "dynamic state" view of HSV persistence. Under this scheme stimuli do not act on persistently infected cells, but rather they alter the local environment of the skin and/or affect the immune response in a way that allows increased viral replication. This amplification of virus titer then would allow detection of otherwise undetectable amounts of virus (10, 38,99).

PART I. PERSISTENT INFECTION WITH BOVINE HERPESVIRUS-1:
A RABBIT MODEL

This manuscript has been submitted to Infection and Immunity

PERSISTENT INFECTION WITH BOVINE
HERPESVIRUS-1: A RABBIT MODEL

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ABSTRACT

Persistent infection with bovine herpesvirus-1 (BHV-1) was established in all rabbits following conjunctival inoculation of virus. Spontaneous reactivations of BHV-1 with and without the appearance of recurrent ocular lesions were observed in persistently infected rabbits. BHV-1 was reactivated predictably and shed from all persistently infected rabbits following the administration of dexamethasone. During all reactivations, BHV-1 isolation was restricted to the inoculated eye.

THE RABBIT MODEL

Bovine herpesvirus-1 (BHV-1) is responsible for a variety of disease conditions in cattle. Included among these are respiratory disease, conjunctivitis, vulvovaginitis, balanoposthitis, meningoencephalitis, and fatal systemic infection (5). Like other members of the herpesvirus group, BHV-1 is capable of establishing persistent infections in its natural host species (1,4,15).

Rabbits have been infected experimentally with BHV-1 and suggested as possible laboratory models for studying the pathogenesis of BHV-1 infection (6,8,9). This study was conducted to determine if rabbits could be persistently infected with BHV-1 and to evaluate the rabbit as a laboratory model for studying persistent BHV-1 infection.

Adult white rabbits (2.5-5.0 kilograms) were purchased from a local commercial source and were housed in individual cages within an animal isolation room. The Cooper strain of BHV-1 was supplied by Dr. A. Strating, National Veterinary Services Laboratory, Ames, Iowa, and was used at the thirteenth passage level. Bovine lung cells were used throughout the experiment and were maintained as previously described (13). Ocular and nasal swabs were collected and immediately immersed in 2.0 ml. of Eagle's minimum essential medium (MEM) containing antibiotics and fungizone (100 I.U. penicillin, 100 µg. kanamycin sulfate, 100 µg. streptomycin sulfate, and 5 µg fungizone per ml.), and vigorously shaken for 15 seconds prior to plating 0.5 ml. onto bovine lung monolayer cell cultures. Cultures were observed daily for the presence of cytopathic effect, and discarded if negative after 7 days. Viral isolates obtained were identified

as BHV-1 using direct immunofluorescence or neutralization with BHV-1 antiserum (9).

Rabbits (N=22) were lightly anesthetized (Metaphane, Pitman-Moore, Inc., Washington Crossing, N.J.) and inoculated by instilling 1.5×10^6 plaque forming units of BHV-1 (in 0.2 ml. MEM) in the right conjunctival sac. Control animals (N=4) were mock-infected with 0.2 ml. MEM in an identical manner.

Four BHV-1 infected rabbits and one non-infected control rabbit were swabbed (right eye, left eye, and nose), for 125 consecutive days to monitor for spontaneous reactivation and shed of BHV-1.

At selected dates post-infection (2-7 months), groups of rabbits were treated with dexamethasone (Azium, Schering Corporation, Kenilworth, N.J.), in an attempt to induce recurrent BHV-1 infection. BHV-1 infected and control rabbits were treated with a four day regimen consisting of daily intramuscular injections of 4 mg. of dexamethasone. In a similar manner a group of rabbits (N=4) received multiple dexamethasone treatments during the course of the experiment.

The acute phase of the infection has been described (9, D. Rock, W. Hagemoser, and D. E. Reed, submitted for publication). Virus was isolated consistently from the right ocular swabs for 9-15 days post-infection. Virus was isolated sporadically from a few animals until 24 days post-infection. Virus was not recovered from the non-infected left eye of BHV-1 infected rabbits or from non-infected control animals during this period. Serum from all inoculated rabbits contained BHV-1 neutralizing

antibody when examined at 30 days post-infection.

Spontaneous reactivation and shed of BHV-1 from the right eye was observed in 3 of the 4 infected rabbits which were continuously monitored (Table 1). Spontaneous reactivation occurred 33 to 104 days post-infection and was characterized by virus shedding for 1-2 days and the absence of clinically apparent infection. However, ocular lesions were observed in one rabbit (418) undergoing spontaneous reactivation. The non-infected control animal monitored in a similar manner was virus-free throughout the observation period.

BHV-1 was isolated from the right eye of all 22 of the previously infected rabbits within 4 days after initiation of dexamethasone treatment (Table 2). Virus was shed for 5-7 days. BHV-1 also was isolated from nasal swabs for 1-3 days. Virus was not isolated from the left eye of any of the treated rabbits. Untreated BHV-1 infected rabbits and dexamethasone treated non-infected control rabbits remained clinically normal and free of virus throughout the treatment period.

The dexamethasone-induced recurrent infection was characterized by a mild to moderate conjunctivitis and the presence of small ulcers on the outer right eye lid (Figure 1). These conditions developed on the last day of dexamethasone treatment and persisted for 6 to 7 days. This recurrent ocular lesion was observed in all rabbits undergoing an initial reactivation with dexamethasone.

Over a period of 15 months, multiple reactivations of BHV-1 in the same rabbit were obtained using dexamethasone (Table 3). Virus was

Table 1. Spontaneous reactivation and shed of BHV-1 from persistently infected rabbits

<u>Rabbit No.</u>	<u>No. of spontaneous recurrent episodes^a</u>	<u>Day post-infection BHV-1 first reisolated</u>	<u>No. of days BHV-1 shed</u>	<u>Observations</u>
414 (non-infected control)	0			
415	1	33 (right eye)	1	eye clinically normal
416	0			
417	1	86 (right eye)	2	eye clinically normal
418	3	70 (right eye)	1	eye clinically normal
		89 (right eye)	1	eye clinically normal
		104 (right eye)	2	small ulcer right eye lid present day 104-108

^aOcular (right and left) and nasal swabs were collected from rabbits for 125 consecutive days post-infection.

Table 2. Reactivation of BHV-1 in dexamethasone treated rabbits:^a initial reactivation

Months post-infection	No. of animals/Total no. reactivated/treated ^b	No. of animals positive/Total no. of animals			Recurrent ocular lesions
		Day post-treatment initiation BHV-1 first reisolated			
		2	3	4	
2	3/3	9/22	12/22	1/22	22/22
3	3/3				
4	3/3				
5	2/2				
6	4/4				
7	7/7				
	<hr/> 22/22				

^aPrior to treatment, one to two weeks of daily attempts to isolate BHV-1 from the right eye, left eye, and nose of infected rabbits were unsuccessful.

^bRabbits were treated with a four day regimen of dexamethasone, consisting of daily intramuscular injections of 4 mg.

Fig. 1. Recurrent ocular lesions in a rabbit persistently infected with BHV-1 7 days after the initiation of dexamethasone treatment. Ulceration of the outer eyelid (arrows) is evident.



Table 3. Reactivation of BHV-1 in dexamethasone treated rabbits:^a multiple reactivations

Rabbit No.	Months post-infection ^b reactivation attempted	Result of reactivation attempt	Day post-treatment initiation BHV-1 first reisolated	No. of days shed	Recurrent ocular lesions
216	3 mo.	+ ^c	2	6	+
	6 mo.	+	3	ND ^d	+
	11 mo.	+	3	6	+
415	4 mo.	+	3	5	+
	7 mo.	+	2	5	+
	11 mo.	+	3	6	+
	15 mo.	+	3	ND	+
416	4 mo.	+	3	6	+
	7 mo.	+	4	3	+
	11 mo.	+	4	3	+
418	4 mo.	+	3	4	+
	7 mo.	+	4	1	-
	11 mo.	+	5	3	-
	15 mo.	-			-

^aRabbits were treated with a four day regimen of dexamethasone, consisting of daily intramuscular injections of 4 mg.

^bPrior to treatment, one to two weeks of daily attempts to isolate BHV-1 from the right eye, left eye, and nose of infected rabbits were unsuccessful.

^cA (+) denotes a positive instance while (-) represents a negative instance.

^dNot determined.

isolated from the right but not the left eye in each case. Subsequent reactivations observed in the rabbits were quite similar to the initial reactivations. A decrease in the duration of virus shed was observed in rabbits 416 and 418 in the later reactivations. Clinically apparent recurrent infection was observed only during the initial reactivation of rabbit 418. An attempt to reactivate rabbit 418 at 15 months post-infection was unsuccessful. Untreated BHV-1 infected rabbits and dexamethasone treated non-infected rabbits were included as controls for each reactivation attempt. Control rabbits remained clinically normal and free of virus throughout each reactivation period.

The results indicate that BHV-1 established a persistent infection in all experimentally infected rabbits. The rabbit model described in this report is consistent with the following observations made on cattle persistently infected with BHV-1. Persistent BHV-1 infection in cattle is characterized by: 1) most, if not all, animals are persistently infected after experimental infection (2,14); 2) spontaneous sporadic isolation of virus without evidence of clinical disease is seen in persistently infected animals (1,4,15); and 3) virus can be reactivated predictably with or without the appearance of clinical disease in approximately 100% of the experimentally infected animals treated with dexamethasone (2,7,14).

Studies on BHV-1 pathogenesis in cattle have suggested that BHV-1 persists in the trigeminal ganglion after intranasal and

intraconjunctival inoculation of virus and is transported centrifugally via the trigeminal nerve to produce recurrent virus shed and disease (3,10,11,12). Lupton, et al. (9) isolated BHV-1 from the trigeminal and optic nerves of rabbits during acute conjunctival infection. The restriction of virus isolation during reactivation of persistently infected rabbits to the ipsilateral inoculated eye (right eye) described in this report, along with the isolation of BHV-1 from rabbit trigeminal ganglia during the persistent phase of the infection (D. Rock. W. Hagemoser, and D. E. Reed; Part II, herein), suggests that the pathogenesis of persistent infection in the rabbit is similar to that thought to occur in cattle. The similarities discussed above suggest the usefulness of the rabbit in the study of persistent BHV-1 infection.

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PART II. THE PATHOGENESIS OF ACUTE AND RECURRENT BOVINE
HERPESVIRUS-1 (INFECTIOUS BOVINE RHINOTRACHEITIS
VIRUS) INFECTION IN RABBITS FOLLOWING CONJUNCTIVAL
INOCULATION

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THE PATHOGENESIS OF ACUTE AND RECURRENT
BOVINE HERPESVIRUS-1 (INFECTIOUS BOVINE
RHINOTRACHEITIS VIRUS) INFECTION IN
RABBITS FOLLOWING CONJUNCTIVAL INOCULATION

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SUMMARY

The pathogenesis of acute and recurrent bovine herpesvirus-1 (BHV-1) infection in rabbits following conjunctival inoculation was studied using virus isolation, fluorescent antibody staining, and histopathologic examination.

Bovine herpesvirus-1 produced a moderate to severe conjunctivitis following conjunctival inoculation. Infection was confined to ocular tissues and the trigeminal ganglion of the inoculated side. Generalization of infection to the central nervous system was not observed.

Recurrent virus shed and ocular disease were induced in persistently infected rabbits following dexamethasone treatment. Recurrent BHV-1 infection, like the acute infection, was confined to ocular tissues and the trigeminal ganglia. Bovine herpesvirus-1 was isolated from the trigeminal ganglia of persistently infected rabbits. In addition, virus-specific fluorescent cells were detected in the trigeminal ganglia of dexamethasone treated rabbits prior to the appearance of virus in ocular tissues. These observations suggest that BHV-1 persists in the trigeminal ganglia of rabbits following conjunctival inoculation.

INTRODUCTION

Bovine herpesvirus-1 (BHV-1) is responsible for a variety of disease conditions in cattle. Included among these are rhinotracheitis, conjunctivitis, vulvovaginitis, balanoposthitis, meningoencephalitis, and fatal systemic infection (Kahrs, 10). Like other members of the herpesvirus group, BHV-1 is capable of establishing persistent infections in its natural host species (Bitsch, 3; Huck, Millar, and Woods, 8; Snowdon, 21).

Rabbits have been infected experimentally with BHV-1 and suggested as possible laboratory models for studying the pathogenesis of BHV-1 infection (Lupton, Barnes, and Reed, 13; Lutpon and Reed, 12; Kelly, 11). Persistent BHV-1 infections have been established in rabbits following conjunctival inoculation of virus. In addition, recurrent virus shed and disease have been induced in these persistently infected rabbits following the administration of dexamethasone (Rock and Reed, 19).

This study was conducted to: (1) examine the pathogenesis of BHV-1 in rabbits following conjunctival inoculation; (2) examine the pathogenesis of recurrent BHV-1 infection in persistently infected rabbits following treatment with dexamethasone; and (3) evaluate the rabbit as a laboratory model for studying persistent BHV-1 infection.

MATERIALS AND METHODS

Cell culture Bovine lung (BLG) and bovine turbinate (BT) cells were grown in Eagle's minimum essential medium with Earle's salts (MEM), supplemented with 10% gamma-irradiated fetal calf serum (FCS), 0.16% sodium bicarbonate, 8 mM N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES), and antibiotics (100 I.U. penicillin, 100 µg streptomycin sulfate, and 100 µg kanamycin sulfate per ml). Cells were grown and maintained at 37 C in a humidified air atmosphere containing 5% carbon dioxide (CO₂).

Virus The Cooper strain of BHV-1 was supplied by Dr. A. Strating, National Veterinary Services Laboratory, Ames, Iowa, and was used at the thirteenth passage level. Stock virus was maintained at -70 C until used.

Experimental animals and inoculations Adult white rabbits (2.5-5.0 kilograms) of either sex were purchased from a local commercial source and were housed in individual cages within an animal isolation room. Rabbits were lightly anesthetized (Metaphane, Pitman-Moore Inc., Washington Crossing, N.J.) and inoculated by instilling 1.5×10^6 plaque forming units of BHV-1 in 0.2 ml MEM in the right conjunctival sac. Control animals were mock-infected with 0.2 ml MEM in an identical manner. Rabbits used in the recurrent infection experiment were inoculated in both the right and left conjunctival sac. All rabbits were free of serum neutralizing antibodies to BHV-1 prior to inoculation.

Experimental design

Acute infection Rabbits (N=18) were inoculated as described above. Three rabbits were killed by electrocution at 2, 5, 8, 11, 14, and 25 days post-inoculation (PI). Samples of various tissues were collected at necropsy for virus isolation (tissue homogenates), histopathology, and fluorescent antibody staining.

A group of rabbits (N=4) were observed throughout the acute and recurrent infection. Ocular and nasal swabs were collected daily during the experimental period.

Recurrent infection Rabbits (N=22) were inoculated bilaterally as described above. Based on the isolation of BHV-1 from ocular swabs at 5 days PI and the presence of neutralizing antibody at 30 days post-infection rabbits were presumed to be persistently infected (Rock and Reed, 1980). At selected dates PI (6-8 months), rabbits were treated with all or some portion of a four day regimen consisting of daily intramuscular injections of 4 mg of dexamethasone (Azium, Schering Corp., Kenilworth, N.J.). Rabbits (N=3) were killed by electrocution at 1, 2, 3, and 6 days post-treatment initiation. Samples of various tissues were collected at necropsy for virus isolation (tissue homogenates and explant culture), histopathology, and fluorescent antibody staining. Non-treated rabbits (N=10) were killed 4-15 months PI and were handled in an identical manner. Prior to dexamethasone treatment (treated animals), or necropsy (non-treated animals), one to two weeks of daily attempts to isolate BHV-1 from ocular and nasal swabs were unsuccessful.

Virus isolation procedures Ocular and nasal swabs were immersed in 2 ml of MEM containing antibiotics and fungizone (100 I.U. penicillin, 100 µg kanamycin sulfate, 100 µg streptomycin sulfate, and 5 µg fungizone per ml) and vigorously shaken for 15 seconds prior to plating 0.5 ml on BLU or BT monolayer cell cultures. The remaining sample was stored frozen at -70 C until titrated. Titrations of swab samples were performed by plaque assay as previously described (Lupton et al., 13).

Tissue samples for virus isolation were processed immediately. Tissue suspensions (10% in MEM) were prepared by homogenization with Ten Broeck tissue grinders. Infectivity was titrated by plaque assay.

Tissue samples for explant culture were washed in MEM, cut into 1-5 mm pieces, and cultured in MEM supplemented with 10% FCS, 5% lamb serum, antibiotics, and fungizone. Cultures were maintained at 37 C in a humidified air atmosphere containing 5% CO₂ for 30 to 45 days, with medium changes every fifth day. Samples of culture fluid were tested for infectivity on BLU or BT monolayers at 5 day intervals. Monolayer cultures were observed daily for the presence of cytopathic effect and were discarded if negative after 7 days. Randomly selected isolates from each animal were identified as BHV-1 using direct immunofluorescence or neutralization with BHV-1 antiserum.

Histopathology and immunofluorescence procedures Tissue samples for histopathology were fixed in 10% neutral buffered formalin and processed for examination using standard paraffin procedures. Sections were stained with hematoxylin and eosin.

Tissue samples for immunofluorescence procedures were frozen in liquid nitrogen and stored at -70 C. Sections were cut at 5 μ , fixed in room temperature acetone for 5 minutes, rinsed for 5 minutes in 0.1 M phosphate buffered saline (PBS), dipped in distilled water, and air dried. Direct fluorescent antibody staining was performed as previously described (Reed, Bicknell, Larson, Knudtson, and Kirkbride, 18). Controls included BHV-1 positive and negative tissue sections as well as the use of a pre-immune conjugate.

In the indirect immunofluorescence procedure, sections were flooded with a hyperimmune bovine anti-BHV-1 serum and incubated for 45 minutes at 37 C in a moist chamber. Sections received two 5-minute rinses in PBS prior to staining for 45 minutes with a commercial conjugate (Cappel Laboratories, Cochranville, Pa.) of fluorescein-labelled rabbit anti-bovine IgG. Sections were rinsed twice in PBS (5 minutes each), once in distilled water (5 minutes), and mounted with buffered glycerol (pH 8.6). Controls included known BHV-1 positive and negative tissue sections. A negative serum control was routinely included.

Electron microscopy Two rabbits were killed by electrocution at 5 days post-inoculation. The trigeminal ganglia were removed immediately and fixed with 3.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.2) for 6 hours at 4 C. Tissues were postfixed with 1% buffered osmium tetroxide, dehydrated with alcohol, and embedded in epon-araldite. Thick sections were stained with a methylene blue-azure II and basic fuchsin stain (Humphrey and Pittman, 9) to orient blocks for thin sectioning. Prior to examination, thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Acute infection All rabbits developed conjunctivitis in the right eye within 48 hours of inoculation. The ocular lesions were most severe 3 to 5 days PI, and were characterized by hyperemia and edema of the conjunctiva, injected scleral vessels, and a serous, or in many cases, a mucopurulent discharge (Fig. 1). A decrease in the severity of the inflammatory response was evident at 6 to 8 days PI. At this time, multiple ulcers were observed on the outer right eyelid of most rabbits (Fig. 2). By 12 days PI, the inoculated right eyes were normal in appearance (Fig. 3). The left eyes of BHV-1 inoculated rabbits and the eyes of mock-infected control rabbits remained clinically normal.

Serum from all 4 of the infected rabbits contained BHV-1 neutralizing antibody when examined at 30 days PI. Serum neutralizing titers ranged from 8 to 32.

Virus isolation Bovine herpesvirus-1 was present in swabs taken from the right eye of all inoculated rabbits on day 1 PI and could be detected for 9-15 consecutive days (Fig. 4). During this period, virus also was isolated from nasal swabs, although with less regularity. Virus was isolated sporadically from a few animals until 24 days PI. Swabs taken from the left eye of inoculated rabbits and from the eyes of mock-infected controls were never found to contain virus.

Figure 1. BHV-1 induced conjunctivitis in a rabbit 4 days after conjunctival inoculation. The rabbit had hyperemia of the conjunctiva, and a mucopurulent discharge.

Figure 2. A rabbit 8 days after conjunctival inoculation of BHV-1. Note the focal ulceration of the outer eyelid (arrows).

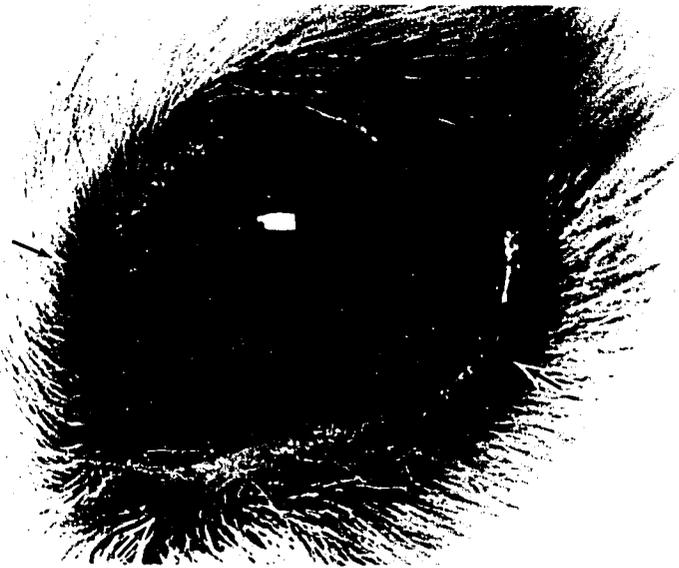
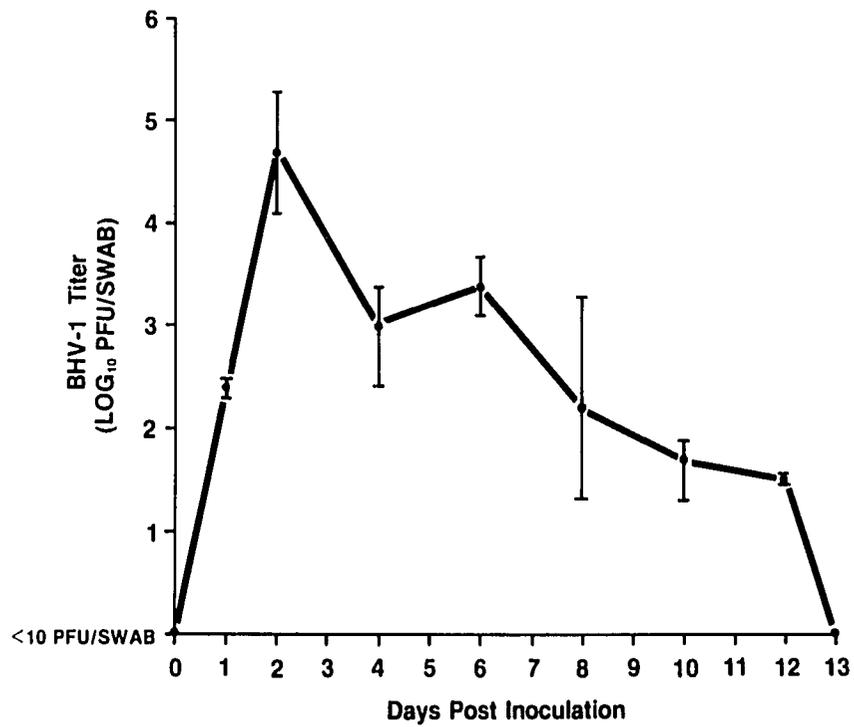
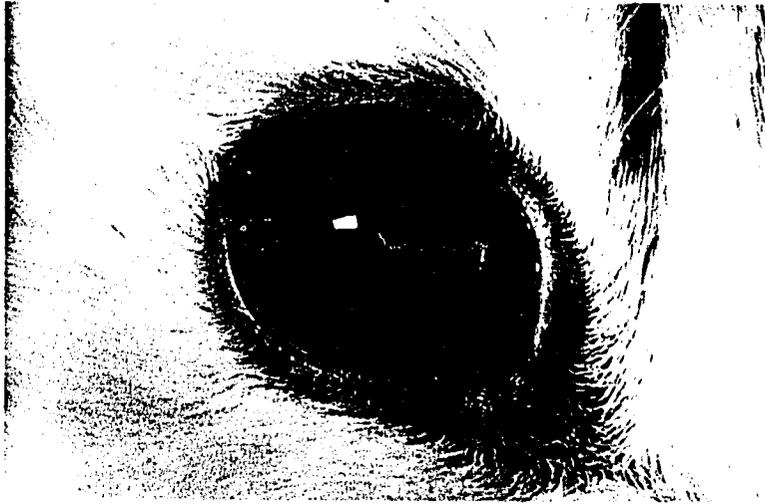


Figure 3. A rabbit 14 days after conjunctival inoculation of
BHV-1. The eye is normal in appearance.

Figure 4. The mean and range of BHV-1 titers in right ocular swabs
from rabbits (N=4) inoculated in the right conjunctival
sac with 1.5×10^6 PFU of BHV-1.



The isolation of BHV-1 was confined to ocular tissues of the inoculated eye and the nasal mucosa (Table 1). Virus titers were the highest in the conjunctiva and cornea, and declined after 5 and 11 days PI respectively. No virus was recovered from tissues examined at 25 days PI. Virus was not recovered from the trigeminal ganglia, pons-medulla, cerebrum, and olfactory bulb using homogenized tissue samples. Using explant culture as a virus isolation procedure, BHV-1 was recovered from the right trigeminal ganglion in 2 of 4 rabbits killed at 5 days PI, but was not recovered from the central nervous system.

Virus was not detected in the medial retropharyngeal lymph node (right and left), adrenal gland, lung, liver, and spleen.

Immunofluorescence and histopathologic examination Bovine herpesvirus-1 specific fluorescence was observed in the right conjunctiva and cornea up to 5 days PI (Table 1). Corneal fluorescence was characterized by focal involvement of the corneal epithelium. In these foci fluorescence appeared to involve the entire epithelial layer (Fig. 5). Focal areas of epithelial fluorescence also were observed in conjunctival sections (Fig. 6). Occasionally, fluorescent cells were seen beneath the surface epithelium in the sub-mucosa.

Specific fluorescence was not observed in the right trigeminal ganglion of animals killed at 2 days PI, but was present in nine of twelve ganglia examined from rabbits killed 5, 8, 11, and 14 days PI. Direct and indirect fluorescence procedures failed to detect evidence of BHV-1 in the ganglia of rabbits killed at 25 days PI. Homogenous

Table 1. Distribution of BHV-1 and virus specific fluorescence in tissues of rabbits following inoculation into the right conjunctival sac^a

Rabbit Number	Days post-inoculation					
	2			5		
	1	2	3	4	5	6
Conjunctiva Right	3.7 ^b	3.5	5.4	1.3	3.6	2.3
Left	-	-	-	-	-	-
Cornea Right	3.8	4.0	3.3	4.6	4.2	4.3
Left	-	-	-	-	-	-
Ventral lacrimal gland Right	-	1.8	2.6	-	4.0	-
Left	-	-	-	-	-	-
Harderian gland Right	2.5	2.0	2.7	-	-	-
Left	-	-	-	-	-	-
Trigeminal ganglion Right	-	-	-	*	*	*
Left	-	-	-	-	-	-
Nasal mucosa	2.2	-	2.3	-	-	-
Ocular swab Right	4.9 ^d	3.7	5.1	3.0	3.5	2.9
Left	-	-	-	-	-	-
Nasal swab	2.3	2.2	3.8	1.8	-	-

^aVirus isolation was attempted using tissue homogenates.

^bLog₁₀ plaque forming units of BHV-1 per gram of tissue.

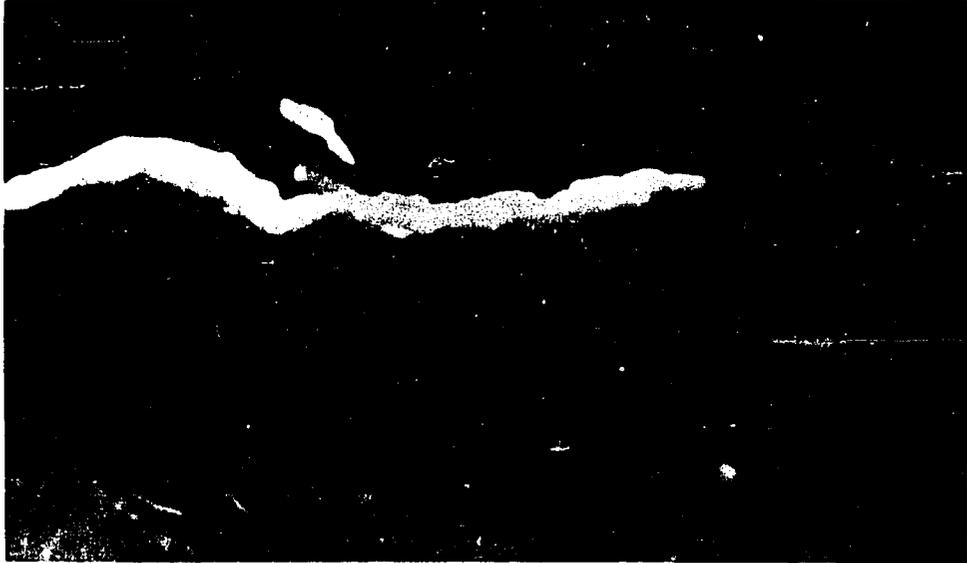
^c(-) indicates a negative sample.

^dSwab values are given as Log₁₀ plaque forming units of BHV-1 per swab.

* Indicates BHV-1 specific fluorescence, negative tissues are unmarked.

Figure 5. Fluorescence microscopy of a section of the right cornea from a rabbit 5 days after conjunctival inoculation of BHV-1. Focal involvement of the entire epithelium is visible. X456.

Figure 6. A focus of BHV-1 specific fluorescence in the epithelial layer of the right conjunctiva is evident in a rabbit killed 2 days after conjunctival inoculation of virus. Fluorescence micrograph X189.



nuclear and granular cytoplasmic fluorescence was seen in involved neurons (Fig. 7). Fluorescence also was observed in satellite cells and small unidentified cells (Fig. 8). Ganglia contained few fluorescent cells and often only one positive cell was seen in a tissue section. No BHV-1 specific fluorescence was observed in the left trigeminal ganglion, pons-medulla, cerebrum, olfactory bulb, medial retropharyngeal lymph node and adrenal gland of inoculated rabbits.

Pathologic changes in the right conjunctiva of animals killed at 2 days PI consisted of focal ulceration of the surface epithelium with a moderate to marked infiltration of heterophils into the base of the ulcers, surface epithelium, and sub-epithelial tissues. A moderate to marked mononuclear cell infiltration of the underlying mucosa also was noted. A decrease in the severity of these lesions was evident by day 5 PI. Mild mononuclear cell infiltration of the sub-mucosa was observed in several animals at 5 days PI or later. In most rabbits examined, mild, diffuse infiltration of heterophils into the surface epithelium was observed in both the inoculated and uninoculated conjunctiva.

Microscopic lesions of the right cornea consisted of focal heterophile infiltration of the epithelium in the area of the limbus. Moderate to marked mononuclear cell infiltration of the sub-epithelium and limbic stroma was observed below areas of surface inflammation. Moderate vascularization and edema were present locally (Fig. 9). These pathologic changes were most severe at 2 and 5 days PI, and milder at 8, 11, and 14 days PI. No lesions were present in the left corneas.

Figure 7. Fluorescence microscopy of a section of the right trigeminal ganglion from a rabbit 5 days after conjunctival inoculation of BHV-1. Nuclear and granular cytoplasmic fluorescence are present in a medium sized neuron. X441.

Figure 8. Fluorescence microscopy of a section of the right trigeminal ganglion from a rabbit 5 days after inoculation of BHV-1. A single unidentified fluorescent cell is visible. X404.

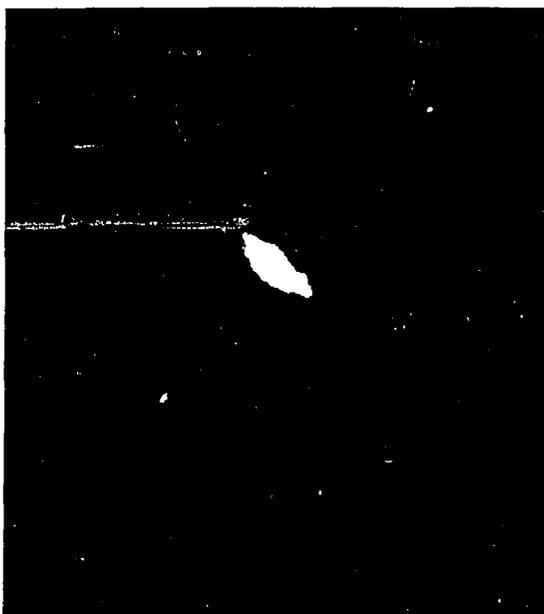
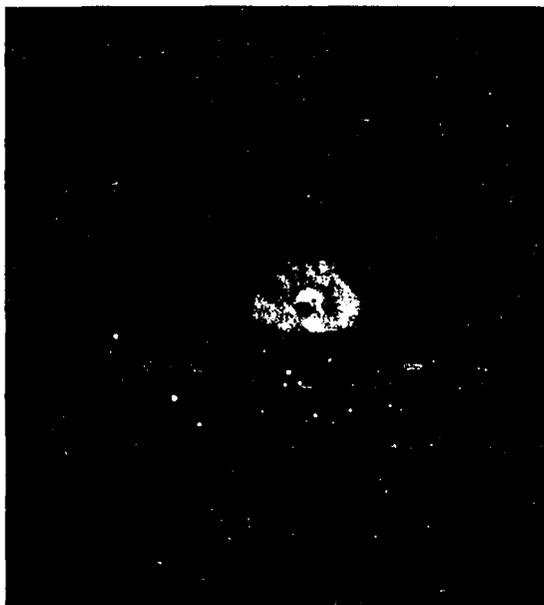
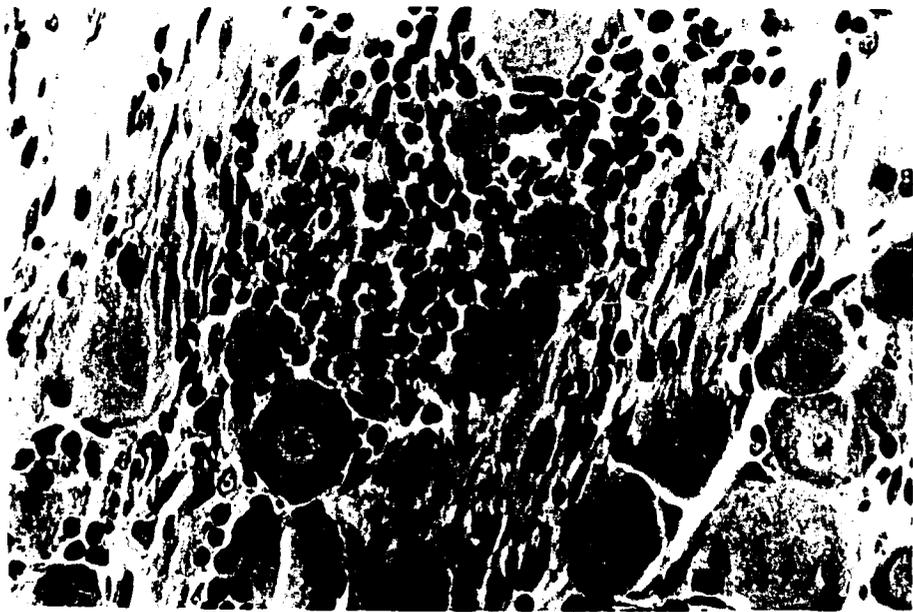


Figure 9. Microscopic changes in the right cornea of a rabbit 5 days after conjunctival inoculation of BHV-1. A moderate mononuclear cell infiltration of the limbic stroma, along with local vascular dilatation and edema are visible. H + E stain; X76.

Figure 10. Focal interstitial mononuclear cell accumulation in the right trigeminal ganglion of a rabbit 5 days after conjunctival inoculation of BHV-1. H + E; X477.



Lesions of the right trigeminal ganglion consisted of focal interstitial mononuclear cell accumulations. Focal neuronal degeneration, neuron necrosis, and neuronophagia were evident to some degree in most ganglia (Fig. 10). These lesions were not seen at 2 days PI but were present in nine of twelve ganglia examined from rabbits killed 5, 8, 11, and 14 days PI. Lesions were present in one of three ganglia examined from animals killed at 25 days PI. No lesions were present in the left trigeminal ganglia.

No significant lesions were observed in the ventral lacrimal gland (right and left), Harderian gland (right and left), adrenal gland, pons-medulla, cerebrum, olfactory bulb, and nasal mucosa.

Electron microscopy of the trigeminal ganglion Foci of inflammation were found in thick sections taken from the trigeminal ganglia of two rabbits killed at 5 days PI. Blocks were trimmed and thin sections were taken from fifteen different areas of inflammation. Thin sections failed to reveal the presence of virions or evidence of viral replication. Areas sampled did contain inflammatory cells and all constituent ganglion cell types.

Recurrent infection The dexamethasone-induced recurrent infection was characterized by a mild to moderate conjunctivitis and the presence of small ulcers on the outer right eyelid (Fig. 11). These conditions developed on the last day of dexamethasone treatment and persisted for 6 to 7 days.

Virus isolation Bovine herpesvirus-1 was present in swabs taken from the right eye of all dexamethasone treated persistently infected rabbits within 72 hours after treatment initiation. Virus was shed from the right eye for 4 to 6 days and was isolated sporadically over this period from nasal swabs (Fig. 12). Virus was not isolated from the uninoculated left eye of treated rabbits. Non-treated persistently infected rabbits and dexamethasone treated uninoculated control animals remained virus-free throughout the reactivation period.

Using explant culture for virus isolation, BHV-1 was recovered from the trigeminal ganglion and the ventral lacrimal gland of non-treated persistently infected rabbits (Table 2). Virus was detected first in explant culture fluids 5 to 25 days after culture initiation. Virus was isolated from two of six trigeminal ganglia taken from rabbits killed at 1 and 2 days post-treatment initiation, but was not recovered from any other tissues. Bovine herpesvirus-1 was isolated both from the trigeminal ganglia and ocular tissues of rabbits killed at 3 and 6 days post-treatment initiation. Virus was never isolated from the pons-medulla, cerebrum, cranial cervical ganglia, nasal mucosa, harderian gland, adrenal gland and medial retropharyngeal lymph node of treated and non-treated persistently infected rabbits or from the trigeminal ganglia of uninoculated control rabbits (N=4).

Immunofluorescence and histopathologic examination Fluorescent cells were detected in the trigeminal ganglia of rabbits killed 2, 3, and 6 days post-treatment initiation (Table 2). Both neurons and smaller unidentified cell types were involved. Like in the acute infection,

Figure 11. Recurrent ocular lesion in a rabbit 7 days after the initiation of dexamethasone treatment. Focal ulcerations (arrows) of the outer eyelid are evident.

Figure 12. The mean and range of BHV-1 titers in right ocular swabs from rabbits (N=3) treated with dexamethasone.

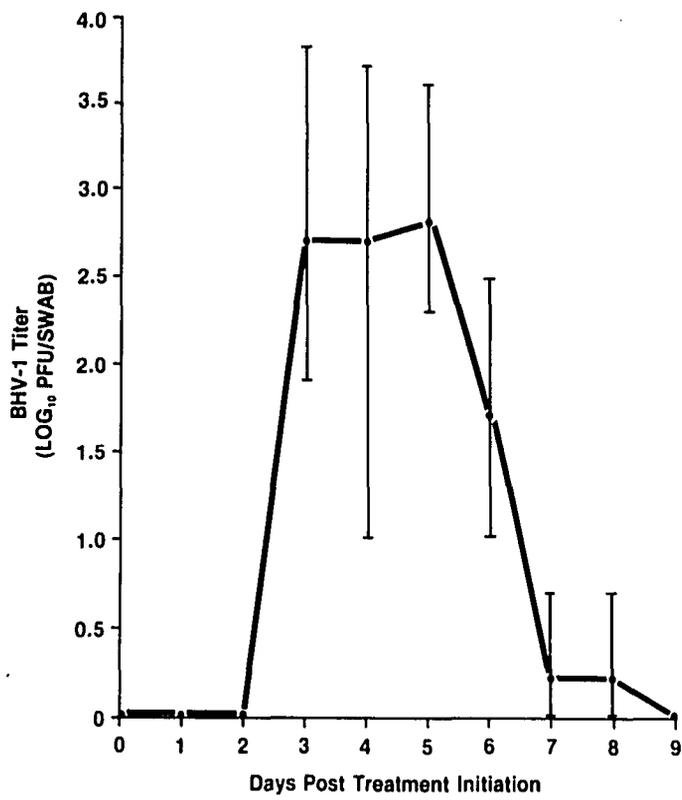


Table 2. Distribution of BHV-1 and virus-specific fluorescence in tissues of persistently infected rabbits following dexamethasone treatment^a

	Non-treated		Days post treatment initiation							
			1		2		3		6	
	Isolation ^b	Fat ^c	Isolation	Fat	Isolation	Fat	Isolation	Fat	Isolation	Fat
Ocular swab	0/10		0/3		1/3		2/3		3/3	
Conjunctiva	0/10	0/7	0/3	0/3	0/3	0/3	1/3	0/3	2/3	0/3
Cornea	0/10	0/7	0/3	0/3	0/3	0/3	1/3	1/3	3/3	0/3
Ventral Lacrimal Gland	1/10	0/7	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3
Trigeminal Ganglia	5/10	0/7	1/3	0/3	1/3	2/3	2/3	2/3	2/3	2/3

^aRabbits were used 4-15 months PI.

^bVirus isolation was attempted using explant cultures (number positive/number examined). Swabs were cultured as described.

^cFluorescent antibody test (number positive/number examined).

there were few fluorescent cells. With a single exception (Table 2), BHV-1 specific fluorescence was not detected in the conjunctiva, cornea, ventral lacrimal gland, harderian gland, nasal mucosa, medial retropharyngeal lymph node and adrenal gland of dexamethasone treated persistently infected rabbits. Bovine herpesvirus-1 specific fluorescence was not observed in tissues taken from non-treated persistently infected rabbits or from treated rabbits 1 day post-treatment initiation.

Corneal lesions consisting of a mild mononuclear cell infiltration below the limbic epithelium were observed in four of seven corneas from non-treated persistently infected rabbits, and in three of twelve corneas examined from rabbits killed 1, 2, 3, and 6 days post-dexamethasone treatment initiation. A more severe lesion was observed in a rabbit killed 6 days post-treatment initiation. The lesion consisted of moderate focal aggregations of lymphocytes with some heterophils beneath the limbic epithelium. Focal ulceration of the corneal epithelium accompanied by epithelial cell swelling and degeneration was also noted. Lesions were not observed in uninoculated control animals.

Pathologic changes in the conjunctiva consisted of a mild to marked heterophile infiltration of the surface epithelium. In addition, a mild to moderate mononuclear cell infiltration of the sub-epithelium was present in some animals. These lesions were present in seven of nine conjunctivas taken from rabbits killed at 2, 3, and 6 days post-treatment initiation. Lesions were most severe at 3 and 6 days post-treatment initiation. With a single exception, lesions were not seen in non-treated persistently infected rabbits. A mild, diffuse infiltration of heterophils

into the surface epithelium was observed in most uninoculated control animals.

Recurrent lesions of the trigeminal ganglia were similar to those observed during the acute infection. Mild to moderate lesions were observed in four of seven non-treated persistently infected animals. The corneal lesions described above were not correlated with the presence of trigeminal lesions in non-treated rabbits. Lesions were seen in one of three ganglia obtained 1 day post-treatment initiation and in eight of nine ganglia examined from rabbits killed 2, 3, and 6 days post-treatment initiation. Lesions were not detected in the trigeminal ganglia of uninoculated control animals.

A lesion of the central nervous system was observed in a non-treated persistently infected rabbit. The lesion, located at the level of the ponto-medullary junction, consisted of a focal mononuclear cell infiltration in the ventral aspect of the fifth nucleus. Moderate pathologic changes also were observed in the trigeminal ganglia of this animal. Central nervous system lesions were not seen in any other treated or non-treated persistently infected rabbits.

Recurrent lesions of the outer eyelid consisted of multiple, ulcerative, necrotic foci with heterophile and macrophage infiltration of the ulcer base. Focal hydropic degeneration of epithelial cells also was noted (Fig. 13). No significant lesions were observed in the ventral lacrimal gland, harderian gland, adrenal gland, and nasal mucosa.

Figure 13. Focal ulceration of the outer eyelid of a rabbit
6 days after the initiation of dexamethasone treatment.
Hydropic degeneration is visible in epithelial cells
adjacent to the ulcerated areas. H + E; X214.



DISCUSSION

The present report demonstrates that BHV-1 produces a moderate to severe conjunctivitis following conjunctival inoculation. The ocular infection described here is similar to those reported by others in rabbits (Lupton et al., 13; Lupton and Reed, 12; Kelly, 11) and cattle (Narita, Inui, Namba, and Shimizu, 16; Davies and Duncan, 5; Abinanti and Plumer, 1).

Acute BHV-1 infection in rabbits differed in several aspects from observations made on experimentally infected cattle. In rabbits, BHV-1 infection was confined to ocular tissues and the trigeminal ganglion of the inoculated side. The generalization of infection that occurs in cattle following peripheral inoculation of virus (Davies and Duncan, 5; McKercher, Wada, and Straub, 14) was not seen in rabbits. The neural involvement observed in BHV-1 infected rabbits also differed from that seen in cattle. In the rabbit, acute infection of the trigeminal ganglion was mild. Virus was recovered from acutely infected trigeminal ganglia only by using explant culture for virus isolation. Immunofluorescence procedures demonstrated few fluorescent cells in acutely infected ganglia, and ultrastructural examination failed to reveal herpes virions or evidence of viral replication. In contrast, BHV-1 has been isolated from the trigeminal ganglia of intranasally inoculated calves up to 15 days PI using tissue homogenates (Narita, Inui, Namba, and Shimizu, 15). Titers of BHV-1 ranging from $10^{2.5}$ to $10^{4.3}$ TCID₅₀ per gram of ganglion tissue have been reported (Bagust and Clark, 2).

Generalization of BHV-1 infection to the central nervous system, a common finding in experimentally infected cattle (Narita et al., 16; Narita et al., 15; Davies and Duncan, 5; McKercher et al., 14), was not seen in rabbits following conjunctival inoculation.

The results indicate that recurrent virus shed and disease could be induced in all BHV-1 persistently infected rabbits following dexamethasone treatment. These findings are similar to those previously reported in rabbits (Rock and Reed, 1981, Part I, herein; Rock and Reed, 19) and cattle (Davies and Carmichael, 4; Sheffy and Davies, 20). In rabbits, recurrent BHV-1 infection was confined to ocular tissues and the trigeminal ganglia.

Bovine herpesvirus-1 was isolated from the trigeminal ganglion in five of ten non-treated persistently infected rabbits. With a single exception (Table 2), virus was not isolated from any other tissues in these animals. In addition, immunofluorescence procedures detected BHV-1 specific fluorescent cells in the trigeminal ganglion of dexamethasone treated rabbits prior to the appearance of virus or virus specific fluorescence in ocular tissues. These findings suggest that BHV-1 persists in the trigeminal ganglion of persistently infected rabbits and is shed to the periphery following dexamethasone treatment. Centrifugal transport of virus via nerves is suggested by: (1) the rapid appearance of virus in ocular tissues following dexamethasone treatment; (2) the restriction of recurrent virus shed and disease to the originally inoculated eye (Rock and Reed, 1980); and (3) the isolation of BHV-1 from

the trigeminal and optic nerves of rabbits following conjunctival inoculation (Lupton et al., 13).

Sensory and autonomic nerve ganglia have been suggested as sites of BHV-1 persistence in infected cattle. Bovine herpesvirus-1 specific fluorescence was detected in the trigeminal ganglia of dexamethasone treated persistently infected calves prior to the appearance of virus in the nasal mucosa (Narita et al., 17). Bovine herpesvirus-1 also has been isolated from the trigeminal ganglia of clinically normal cattle at slaughter (Homan and Easterday, 6), although prior attempts by other researchers to isolate virus from ganglia of non-dexamethasone treated experimentally infected cattle were unsuccessful (Davies and Duncan, 5; Davies and Carmichael, 4). Recently, Homan and Easterday (7) have demonstrated the presence of BHV-1 DNA in neurons of the trigeminal and superior cervical ganglia of persistently infected calves.

Pathologic changes, consistent with those seen in recurrent BHV-1 infection, were detected in four of seven trigeminal ganglia examined from non-treated persistently infected rabbits. Bovine herpesvirus-1 eventually was isolated from three of the four ganglia, although no virus specific fluorescence was detected in any of the ganglia at the time of explant culture. Spontaneous reactivation of BHV-1 has been described in persistently infected rabbits (Rock and Reed, 19) and may provide an explanation for this observation.

Unlike observations made on persistently infected cattle following dexamethasone treatment (Narita et al., 17; Davies and Duncan, 5), evidence suggesting central nervous system involvement in recurrent BHV-1

infection was not seen in rabbits. However, histopathologic lesions of the brain stem, consistent with those seen in BHV-1 infections of cattle (Narita et al., 16; Narita et al., 17), were observed in a single non-treated persistently infected rabbit. Virus or virus specific fluorescence was not detected in the brain stem of this animal. This observation suggests that, although not characteristic, central nervous system involvement may occur in adult rabbits under certain conditions.

Persistent BHV-1 infection in rabbits is characterized by: (1) most, if not all, animals are persistently infected following conjunctival inoculation (Rock and Reed, 19); (2) spontaneous sporadic isolation of virus with or without evidence of clinical disease is seen in persistently infected animals (Rock and Reed, 19); and (3) virus can be reactivated predictably from experimentally infected animals following dexamethasone treatment (Rock and Reed, 19). These observations are consistent with those made on BHV-1 infected cattle (Snowdon, 21; Sheffy and Davies, 20). The similarities described above in conjunction with the similarities in pathogenesis described in this report suggest the usefulness of the rabbit as a laboratory model for the study of persistent BHV-1 infections of cattle.

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SUMMARY AND CONCLUSIONS

This study consisted of two parts: (1) an examination of the rabbit as a laboratory model for the study of persistent bovine herpesvirus-1 infection (BHV-1) and (2) an examination of the pathogenesis of acute and recurrent BHV-1 infection in rabbits following conjunctival inoculation.

Persistent infection with BHV-1 was established in all rabbits following conjunctival inoculation of virus. Spontaneous reactivations of BHV-1 with and without the appearance of recurrent ocular lesions were observed in persistently infected rabbits. BHV-1 was reactivated predictably and shed from all persistently infected rabbits following the administration of dexamethasone. Recurrent virus shed and ocular lesions were restricted to the inoculated eye.

The pathogenesis of acute and recurrent BHV-1 infection in rabbits following conjunctival inoculation was studied using virus isolation, fluorescent antibody staining, and histopathologic examination. BHV-1 produced a moderate to severe conjunctivitis following conjunctival inoculation. Infection was confined to ocular tissues and the trigeminal ganglion of the inoculated side. Generalization of infection to the central nervous system was not observed.

Recurrent BHV-1 infection, induced following dexamethasone treatment, was confined to ocular tissues and the trigeminal ganglia. BHV-1 was isolated from the trigeminal ganglia of persistently infected rabbits. In addition, virus specific fluorescent cells were detected in the trigeminal ganglia of dexamethasone treated rabbits prior to the appearance

of virus in ocular tissues. These observations suggest that BHV-1 persists in the trigeminal ganglia of rabbits following conjunctival inoculation.

In conclusion, the rabbit model of persistent BHV-1 infection described in this study faithfully reproduces many aspects of the persistent infection observed in cattle, and should prove useful in the study of persistent BHV-1 infection.

A more detailed understanding of this laboratory model, and BHV-1 persistence in general, would be provided by the following research:

1. Research is needed to determine what ganglion cell types are infected following conjunctival inoculation of rabbits with BHV-1. Attempts should be made to determine if all cell types are permissive for viral replication.
2. Trigeminal ganglia from persistently infected rabbits should be examined to determine what cell types harbor latent virus, and what state of activity the viral genome is in. Experiments utilizing nucleic acid hybridization could be used to answer both of these questions.
3. Further study is needed to elucidate the mechanism of dexamethasone induced reactivation of persistently infected animals.
4. Additional research is needed to determine if the natural course of persistent BHV-1 infection can be modulated. More specifically, can immunologic or physiologic manipulations prevent the establishment of persistent infections in non-infected animals, or prevent the reactivation and shed of virus in

animals already persistently infected? Preliminary studies could be conducted easily in rabbits.

5. The absence of central nervous system involvement in adult rabbits during acute or recurrent BHV-1 infection should be investigated further.

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