CASE REPORT

Acute primary canine herpesvirus-1 dendritic ulcerative keratitis in an adult dog

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Abstract
We present a report of dendritic ulcerative keratitis in a 4-year old locally immunosuppressed dog suspected to result from acute primary canine herpesvirus-1 (CHV-1) infection. The dog was presented for evaluation of mild blepharospasm and conjunctival hyperemia in the right eye (OD) shortly after attending a public boarding facility. For approximately 3 months, the dog had been receiving topical prednisolone acetate 1.0% and tacrolimus 0.02% in both eyes (OU) q12h for treatment of follicular conjunctivitis. Ophthalmic examination revealed three regions of corneal fluorescein retention OD. The lesions had a dendritic pattern, were approximately 2–3 mm in length, and were located at the dorsomedial, lateral, and ventromedial aspects of the cornea. No additional abnormalities were noted on complete ophthalmic and physical examinations. CHV-1 was identified in conjunctival samples OD by polymerase chain reaction, and paired CHV-1 serum virus neutralization antibody titers were positive and consistent with acute infection. Topical prednisolone acetate and tacrolimus were discontinued. The dog was treated with cidofovir 0.5% OU q12h for a period of 4 weeks, with resolution of corneal disease noted within 1 week of treatment. In conjunction with previous studies, this case report supports a central role for alterations in host immune status in the pathogenesis and clinical manifestations of CHV-1 ocular disease in dogs.

Key Words: canine herpesvirus-1, cidofovir, corticosteroid, dendritic ulcerative keratitis, tacrolimus

INTRODUCTION

Canine herpesvirus-1 (CHV-1) is a member of the Varicellovirus genus of the herpesvirus subfamily Alphaherpesvirinae, for which one serotype has been recognized.1 CHV-1 occurs worldwide in both domestic and wild dogs, and naturally acquired infection is common with seropositivity >90% in some populations.2–4 CHV-1 is antigenically and biologically related to other alphaherpesviruses including feline herpesvirus-1 (FHV-1) and herpes simplex virus-1 (HSV-1).5,6 In this group of related viruses, clinical features and severity of disease are often dependent on patient age. While primary CHV-1 infection of fetal and neonatal canines results in severe systemic disease which is often fatal,7,8 primary infection of adult dogs is typically subclinical or limited to localized respiratory and genital disease.9–11 Alphaherpesviruses including CHV-1 possess the ability to establish lifelong latency within host lymphatic tissue and neurons of sensory ganglia following primary infection. Reactivation of latent virus during periods of host immunosuppression results in active viral shedding and may be asymptomatic or associated with recrudescent disease.12–14 Ocular surface disease is a well-documented manifestation of FHV-1 and HSV-1 infection in cats and humans, respectively. In these species, the majority of ocular morbidity in adult hosts is a result of recrudescent disease associated with viral reactivation, which can be severe and cause permanent visual impairment.15,16 While less commonly recognized in canines, naturally occurring cases of both primary and recrudescent ocular disease associated with CHV-1 infection have been reported,17,18 and disease has also been induced experimentally.19,20 Clinical features of CHV-1 ocular disease are similar to those associated with alphaherpesviruses in other species and involve conjunctivitis and nonulcerative and

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ulcerative keratitides.\textsuperscript{17–21} Dendritic ulcerative keratitis is a pathognomonic lesion associated with alphaherpesvirus ocular infection\textsuperscript{22,23} and results from viral replication in the basal cell layer of the corneal epithelium.\textsuperscript{15}

During primary ocular viral infection, multiple factors contribute to viral clearance including components of host innate (e.g., tear film components, the corneal epithelial barrier, and innate inflammatory cells) and adaptive (e.g., humoral and cellular) immunity.\textsuperscript{24} Alterations in host immune status are associated with increased risk of alphaherpesvirus-induced ocular disease in dogs and in other species. Such alterations may be the result of concurrent disease states, but may also be iatrogenic and related to therapeutic use of common immunosuppressive medications.\textsuperscript{15,16,18,20,25}

The high seroprevalence of CHV-1 and relatively low reported incidence of ocular morbidity in dogs compared with other species affected by analogous alphaherpesviruses suggest that unique viral and host factors contribute to pathogenesis and clinical manifestations of CHV-1 ocular disease in dogs. Below, we present a case description of acute dendritic ulcerative keratitis suspected to result from primary CHV-1 ocular infection in a locally immunosuppressed adult dog.

**CASE REPORT**

A 4-year-old male neutered Labrador retriever was presented for evaluation of a 24- to 36-h history of conjunctival hyperemia and blepharospasm in the right eye (OD). The dog had a 12- to 15-month history of follicular conjunctivitis in both eyes (OU), which at the time of presentation was being treated topically with prednisolone acetate 1.0% OU q12h (Falcon Pharmaceuticals Ltd, Fort Worth, TX, USA) and tacrolimus 0.02% OU q12h (Wedgewood Pharmacy, Swedesboro, NJ, USA). This treatment regimen had been in place for approximately 3 months with follicular conjunctivitis well controlled during that time. Prior to that time, the dog was treated with multiple 8-week tapering courses of neomycin/polymyxin B/dexamethasone ophthalmic ointment OU (Falcon Pharmaceuticals Ltd). The dog had no other significant prior medical history with the exception of dermatologic signs (e.g., pruritus, erythema, recurrent otitis) potentially consistent with atopy. The dog had received systemic prednisone for these signs at one time ending approximately 2 years prior, however was not receiving any systemic medications at the time of presentation. According to the owner, the dog had never displayed signs of upper respiratory infection and had been adopted at approximately 1.5 years of age, with limited information available regarding his prior health status. The dog was recently housed at a public boarding facility for 4 days and returned home approximately 4 days prior to presentation.

On initial presentation, mild blepharospasm and conjunctival hyperemia were noted OD. Ophthalmic examination revealed a positive menace response, dazzle reflex, and intact direct and consensual pupillary light reflexes OU. The pupils were mid-range and symmetrical OU. Schirmer tear test values were normal OU (15 mm/30 s). Intraocular pressures were also normal at 17 mmHg OU (Tono-Pen Vet; Reichert Corp., Depew, NY, USA).

Three regions of corneal fluorescein retention were noted OD. These lesions were not visible unstained and did not appear to involve the corneal stroma to any significant extent. The lesions had a dendritic pattern, were approximately 2–3 mm in length, and were located at the dorsomedial, lateral, and ventromedial aspects of the cornea (Fig. 1). The surrounding cornea was clear, with no evidence of edema, inflammatory infiltrates, or neovascularization. Fluorescein and Rose Bengal staining were negative in the left eye (OS), and aqueous flare was not observed in either eye. Examination of the posterior surface of the nictitating membrane and conjunctival fornices revealed no retained foreign material, and no follicles were observed OU. The remainder of the ophthalmic examination including slit-lamp biomicroscopy (SL-15; Kowa Ltd., Tokyo, Japan) and indirect ophthalmoscopy (Welch Allyn binocular indirect ophthalmoscope; Welch Allyn Medical Products, Skaneateles Falls, NY, USA) was unremarkable. The dog was afebrile at the time of presentation, and no additional abnormalities were detected on physical examination including presence of nasal discharge or other clinical evidence of upper respiratory infection.

Figure 1. Clinical photograph of the right eye (OD) following administration of topical sodium fluorescein. Note the region of stain retention in the lateral paraxial cornea, demonstrating a dendritic pattern. Two other regions of stain uptake with a similar appearance were also identified in the dorsomedial and ventromedial aspects of the cornea OD.
Pharma, Dollard des Ormeaux, Quebec, Canada) were also prescribed. Topical prednisolone acetate and tacrolimus were discontinued OU. Tobramycin was subsequently discontinued after 1 day of treatment owing to a complaint of increased redness and blepharospasm OU following administration of this medication.

At the time of presentation, a conjunctival swab specimen was collected OD for CHV-1 PCR, results of which were positive. Furthermore, blood was collected and serum was obtained for a serum virus neutralization antibody titer for CHV-1. The antibody titer was positive at 1:96, which represents a marked elevation consistent with acute exposure. All diagnostic testing was performed at the College of Veterinary Medicine at Cornell University as previously described.26

One week after initial presentation, the dog was reevaluated. The owner reported resolution of blepharospasm OD, with mild conjunctival hyperemia OU. Ophthalmic examination revealed mild chemosis OD and mild conjunctival hyperemia OU (OS > OD), which were primarily limited to the lower palpebral conjunctiva. Fluorescein and Rose Bengal staining were negative OU. Retraction of the nictitating membrane revealed mild follicle formation OU (OS > OD). The remainder of the ophthalmic and physical examinations were unchanged from the initial evaluation. Topical cidofovir and lubrication were continued at the current dosing schedule for the next 3 weeks. Four weeks after initial presentation, a paired serum virus neutralization antibody titer for CHV-1 was analyzed concurrently with the first serum sample. Results were again positive at 1:42, which represents a modest decline in antibody titer over the course of 4 weeks and is most consistent with acute infection.19

During the treatment period, the owner complained of persistent mild conjunctival hyperemia and ocular discharge OU. Weekly ophthalmic examinations revealed persistent mild follicular conjunctivitis OU, with no fluorescein or Rose Bengal uptake noted at any time. The above clinical signs were attributed to a relapse of follicular conjunctivitis as opposed to a manifestation of herpetic disease based on the reappearance of follicles shortly following discontinuation of topical prednisolone acetate and tacrolimus, as well as lack of evidence of corneal disease during the period of antiviral treatment. Prednisolone acetate was reinstituted OU q12h 3 weeks after the initial presentation, with the first week coinciding with the last week of treatment with cidofovir. Significant resolution of follicular conjunctivitis was not achieved after 2 weeks of treatment with prednisolone acetate. Cromolyn sodium 4% OU q8h (Falcon Pharmaceuticals Ltd) was then instituted, which resulted in clinical improvement of follicular conjunctivitis after 1 week of treatment, at which time the prednisolone acetate was tapered. Clinical evidence of corneal disease (e.g., blepharospasm, conjunctival hyperemia, corneal ulceration, and neovascularization) was not noted during this time period, and no new ocular or physical examination abnormalities were identified.

DISCUSSION

Although more commonly associated with recrudescent disease, dendritic ulcerative keratitis has been recognized as a component of primary alphaherpesvirus ocular infection in affected host species including canines. Prevalence of this lesion during primary infection ranges from 20% to 25%.17,27,28 In this case report, we document dendritic ulceration along with evidence of acute CHV-1 infection in an adult, locally immunosuppressed dog. Spontaneous subclinical ocular shedding of alphaherpesviruses has been documented in cats and humans infected with FHV-1 and HSV-1, respectively.29,30 While subclinical ocular shedding of CHV-1 has been shown to occur in dogs during experimentally-induced viral reactivation,14 it has not been recognized in dogs with CHV-1 ocular disease occurring under natural conditions.21 Thus, the appearance of classic dendritic lesions in conjunction with a positive CHV-1 PCR assay, positive serum virus neutralization antibody titers, and positive response to antiviral treatment is strongly suggestive of a causal role for CHV-1 in this dog’s corneal disease, and the identification of this virus is unlikely to represent an incidental finding.17

The history, clinical presentation, and diagnostic data for the reported case are most suggestive of acute primary CHV-1 infection, as opposed to recrudescent ocular disease. Transmission of CHV-1 occurs via direct contact with infected oronasal, ocular, and genital secretions shed by carriers as the virus is unstable and quickly inactivated in the environment.9 The dog came into close contact with other dogs at a boarding kennel shortly before presentation, which provides a probable source for exposure. The high seroprevalence of CHV-1 in various canine populations suggests that contact with a dog actively shedding the virus in this type of environment is likely. Direct ocular inoculation of dogs with CHV-1 results in marked elevation in serum neutralization antibody titers within 7–14 days, after which titers decline slowly and persist for up to 8 months.19 In the reported case, the marked elevation in antibody titer shortly after presentation and modest decline following resolution of ocular disease are consistent with those described in the previous study and thus support the conclusion that acute primary CHV-1 ocular infection occurred. In contrast, CHV-1 reactivation results in a blunted but faster rise in antibody titers compared with acute infection, with a more rapid decline following resolution of ocular disease.20

In a clinical report of an isolated population of dogs affected by an acute ocular outbreak of CHV-1, ulcerative keratitis occurred in 26% of dogs, and 71% of these ulcers were dendritic in nature.17 Dendritic ulcerative keratitis has also been described in two dogs with naturally acquired CHV-1 recrudescent disease.18 However, in an experimental study of primary CHV-1 in dogs, direct ocular inoculation of the virus did not result in detectable corneal ulceration of any kind.19 The apparent discrepancy between these reports with respect to the occurrence of corneal

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ulceration suggests that multiple complex factors influence the pathogenesis and clinical manifestations of naturally occurring CHV-1 ocular disease. The disease course and pathogenicity of other alphaherpesviruses are mediated by both virus factors (e.g., virus strain and pathogenic factors) and host factors (e.g., innate immunity as dictated by host genetic makeup; local and systemic immunosuppression; corneal epithelial integrity), and the specific influence of these factors on the ocular pathogenicity of CHV-1 is not completely understood. In conjunction with previous reports, the occurrence of dendritic ulcerative keratitis in the dog of this report further supports the conclusion that this lesion should be considered a possible manifestation of primary ocular CHV-1 disease, particularly in a patient with increased susceptibility because of endogenous or exogenous factors. Local immunosuppression may play a central role in allowing the establishment of CHV-1 ocular infection that results in clinically evident ocular disease in the form of corneal ulceration. The reported topical corticosteroid and tacrolimus use in the dog described in this report are likely relevant to the development of disease.

Administration of systemic corticosteroid to CHV-1 latently infected dogs reliably results in active viral shedding and clinically detectable recrudescent ocular disease. Various clinical reports have identified topical ocular corticosteroid use as a risk factor for the occurrence of alphaherpesvirus-associated ocular disease, though experimental models have not been able to confirm the link in dogs. Subconjunctival administration of methylprednisolone acetate did not worsen clinical ocular disease scores in dogs acutely infected with CHV-1 via direct ocular inoculation, and topical prednisolone acetate administration did not result in detectable reactivation of CHV-1 ocular disease in another study group. However, as noted above, the pattern of disease in experimentally infected dogs may differ significantly from that seen in naturally infected animals. Documented clinical associations between topical steroid use and alphaherpesvirus-associated keratitis, including the present case report, strongly suggest that a pathophysiologic link exists, though specific mechanisms remain to be elucidated. In humans, glucocorticoids have recently been shown to inhibit innate corneal epithelial immunity via downregulation of IFN-β production, thus increasing susceptibility of human corneal epithelial cells to HSV-1 infection and replication. A unique feature of the present case report is the history of prolonged topical ocular corticosteroid use over the course of several months, which may have resulted in alteration in corneal immunity to a sufficient degree to permit CHV-1 ocular infection in a naïve adult animal. Ultimately, topical corticosteroid use should be considered a risk factor for CHV-1 ocular disease, and the specific mechanisms underlying this relationship warrant further study.

Tacrolimus is a potent immunosuppressive medication that inhibits growth and proliferation of T-lymphocytes via inhibition of production of interleukin-2 (IL-2). The mechanism of action of tacrolimus is similar to that of cyclosporine, although tacrolimus has been shown to be up to 100 times more potent in its ability to inhibit IL-2 mRNA synthesis. Recognition and clearance of virally infected cells by cytotoxic T-lymphocytes represents an important component of host immune response to viral infection. A clinical association between topical ocular tacrolimus or cyclosporine use and alphaherpesvirus-associated dendritic epithelial keratitis has been described in isolated case reports in humans and canines, in which the continued use of these medications was thought to have contributed to delayed resolution of dendritic ulcers even with concurrent antiviral treatment. Additionally, in one reported case of HSV-1 keratitis associated with topical tacrolimus use, the patient had no prior history of HSV-1 infection, suggesting that the drug may have increased host susceptibility to primary infection. These reports suggest that the immunosuppressive effects of topical tacrolimus may be sufficient to impair viral clearance and inhibit innate defense mechanisms, resulting in clinically relevant epithelial keratitis during primary or recrudescent ocular infection. We believe that topical tacrolimus use may have significantly predisposed the dog described in this report to primary ocular CHV-1 infection and clinically relevant corneal disease.

In addition to immunosuppression via exogenous administration of medications, endogenous alterations in host immune status may also contribute to the pathogenesis of CHV-1 ocular disease. In two previously described clinical occurrences of recrudescent CHV-1 ulcerative keratitis, both dogs were being treated for underlying systemic diseases associated with immune system abnormalities (e.g., immune-mediated thrombocytopenia and diabetes mellitus). Interestingly, an increased susceptibility of atopic human patients to more severe HSV-1 keratitis has been described, possibly reflecting altered T-cell function in these patients. The dog of this report had a history of follicular conjunctivitis and allergic dermatitis consistent with atopy, suggesting potential intrinsic alterations in immune function. These too may have contributed to the development of primary CHV-1 epithelial keratitis in this dog.

The case described above highlights several risk factors that may have an association with CHV-1-induced ocular disease. Although ocular disease in dogs is less commonly reported in comparison with alphaherpesvirus-associated ocular disease in other species, CHV-1 nevertheless represents a clinically important entity. In this regard, important factors to consider include the high prevalence of latent CHV-1 infection in canine populations, as well as the increasing number of dogs living longer with significant comorbidities and/or receiving topical and systemic immunosuppressive medications. Additional research is warranted to further elucidate viral and host factors contributing to ocular pathology associated with CHV-1.

Finally, to the authors’ knowledge, this is the first report of a positive response to twice-daily application of 0.5% cidofovir in naturally occurring primary CHV-1 ocular infection. Resolution of dendritic ulceration and improve-
ment in clinical signs occurred within 7 days of initiation of treatment, and treatment was continued for 1 month after presentation with no recurrence of corneal disease. Additionally, concurrent topical administration of prednisolone acetate during the last week of treatment with cidofovir did not appear to alter the course of corneal healing, cause a relapse of herpetic corneal disease, or result in any other adverse effects. Cidofovir has recently been found to be an effective topical antiviral medication in experimental primary FHV-1 ocular disease in cats when applied twice daily. The long half-life and extended persistence of cidofovir in ocular tissues allow less frequent administration and shorter duration of treatment as compared with other antiviral medications. Because experimental, clinical, and in vitro evidence supporting the efficacy of cidofovir against CHV-1 are currently lacking, the possibility of spontaneous resolution of herpetic corneal disease in the present case report must also be considered. Nevertheless, the apparent positive response to treatment in this case suggests that 0.5% cidofovir may be an effective treatment for CHV-1 ocular disease in dogs when administered twice daily. Further study is needed to evaluate the pharmacokinetics and efficacy of this drug in dogs.

REFERENCES


