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CORTICOSTEROID TREATMENT DOES NOT REACTIVATE CANINE HERPESVIRUS IN RED FOXES

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ABSTRACT: To study canine herpesvirus (CHV) reactivation from red foxes (*Vulpes vulpes*), 29 foxes with varying CHV antibody and CHV carrier status were treated with methylprednisolone acetate, a glucocorticosteroid drug with prolonged immunosuppressive effect in dogs. In the first experiment, 17 foxes with unknown CHV carrier status were treated once with methylprednisolone; in the second experiment, five foxes were treated twice, 4 mo after being intravenously CHV infected; and in the third experiment, six foxes were treated five times, 11 mo after peroral CHV infection. Infectious CHV was not isolated after treatment from either naturally or experimentally CHV-infected foxes or from untreated, CHV-seronegative in-contact foxes. Canine herpesvirus DNA was not detectable in mucosal secretions or white blood cells of any of the foxes, whereas all trigeminal ganglia of experimentally CHV-infected foxes were polymerase chain reaction-positive. In CHV-seropositive foxes, anti-CHV antibody titers did not change with time after treatment, and CHV-seronegative in-contact controls did not seroconvert. Hematologic parameters remained mostly unchanged. We conclude that CHV is not as easily reactivated in foxes following corticosteroid treatment as in dogs, although there was no obvious sign of immunosuppression. Canine herpesvirus was not spread from virus carriers to naive in-contact foxes, which may be among possible explanations for the reported low CHV prevalence in wild foxes.

Key words: Canine herpesvirus, depomedrol, fox, immunosuppression, methylprednisolone, latency, *Vulpes vulpes*.

INTRODUCTION

Red foxes (*Vulpes vulpes*) succumb to most virus infections commonly described in domestic dogs, including canine herpesvirus (CHV; Davidson et al., 1992; Garcelon et al., 1992; Truyen et al., 1998; Robinson et al., 2003). Canine herpesevirus is a member of the α-herpesvirus subfamily that can cause severe hemorrhagic disease in neonatal pups and mild or subclinical respiratory infections in adult dogs (Appel, 1987). Canine herpesvirus is also known to establish latent infections in dogs that can be readily reactivated by immunosuppressive drug treatment (Okuda et al., 1993a, b). Recurrent episodes of CHV shedding are known to be common in dogs and frequently occur without clinical signs of disease (Okuda et al., 1993a). The potential of herpesvirus reactivation and shedding following periods of latency plays a crucial role in the epidemiology of these viruses (Whitley, 1996).

There is no evidence from the literature that CHV causes latent infections in foxes. It is also not known whether infectious virus can be reactivated during immunosuppressive conditions such as stress caused by mating, lactation, food or water shortage, or opportunistic infectious diseases. Seroprevalence of CHV infections in red foxes in Australia is low (around 3%; T. Robinson, pers. comm.), which differs from other viral infections of foxes such as canine adenovirus or canine parvovirus infections, which can infect up to 50% of the population in some locations (Davidson et al., 1992; Garcelon et al., 1992; Truyen et al., 1998). Reasons for this variance are not known but may either be related to the properties of the virus (for example virulence for foxes or the potential for latent infection and reactivation in this species) or to the socioecologic interactions of foxes, which may affect the epidemiology of CHV in foxes differently than in other canines such as dogs or coyotes (*Canis latrans*).

The most significant effect of glucocorticosteroids on the immune system is immunosuppression of peripheral T lymphocytes, which is due to interference with a

wide variety of activation-induced cellular gene products, mainly with cytokines. Glucocorticosteroids also induce apoptosis and cell death of thymocytes (Ashwell et al., 2000). These immunosuppressive functions are widely appreciated in therapeutic treatment of autoimmune and inflammatory conditions. However, they inadvertently can also cause unwanted reactivation of latent virus infections in a variety of medical conditions (Lee et al., 1998; Emery et al., 2000; Kornberg et al., 2001).

Experimentally, corticosteroids are frequently used to deliberately suppress cell-mediated immunity to allow reactivation and shedding of viruses, which cause latent infections such as herpesviruses (Jordan et al., 1977; Edington et al., 1985; Rabinovitch et al., 1990; Guliani et al., 1999; Maggs et al., 2003). Methylprednisolone acetate (depomedrol, Upjohn, Kalamazoo, Michigan, USA) is a glucocorticosteroid with a prolonged effect and known immunosuppressive properties for dogs and cats. It is derived from prednisolone, which has been used to induce reactivation of latent herpesvirus infections in a variety of animals including cats (Kruger et al., 1990; Reubel et al., 1993; Hickman et al., 1994; Maggs et al., 2003) and dogs (Okuda et al., 1993a, b). Its use is also described in foxes to manipulate the distribution of rabies virus following vaccination (Ciuchini et al., 1986).

Canine herpesvirus is currently being investigated as a potential bait-delivered vaccine vector for wild canids. In a previous study, we addressed aspects of virulence and epidemiology of CHV infection in red foxes in Australia (Reubel et al., 2001). From this study and from anecdotal evidence it appears that CHV is not readily passed on from infected to naive foxes. Knowledge of vaccine virus shedding by bait-vaccinated foxes is crucial for optimizing vaccination strategies. Potential spread of vaccine virus into the environment or between individuals may have implications on the appropriate design of the recombinant vaccine virus (for example the desired degree of virus attenuation) or on the nature of the baiting strategy (self-spreading vaccine or bait delivery).

In the present study we explored whether treatment of CHV-seropositive foxes with a corticosteroid drug would result in shedding of infectious CHV. Three experiments were carried out. In the first, we selected CHV-seropositive and CHV-seronegative foxes with unknown CHV carrier status, while in the second and third experiments, experimentally CHV-infected foxes were used. In the latter two experiments these foxes were paired and housed together with sentinel CHV-seronegative foxes to monitor potential virus spreading.

MATERIAL AND METHODS

Animals

Twenty-nine adult captive red foxes (14 males, 15 females, aged 1–3 yr) were selected for the study. Foxes were purchased as cubs from farmers throughout Victoria and New South Wales (Australia) and reared in the animal facilities of Commonwealth Scientific and Industrial Research Organisation (CSIRO) Sustainable Ecosystems in Canberra, Australian Capital Territory. The project was approved by CSIRO Sustainable Ecosystems' Animal Ethics Committee (approval numbers 98/99-20 and 99/00-02). All foxes were vaccinated against canine distemper virus, canine parvovirus, and canine parainfluenza virus with a commercially available vaccine (Protech™C3, Webster, Castle Hill, Australia). Foxes were kept in outdoor cages equipped with wooden shelter boxes, fed daily with canned and dry dog food, and had unrestricted access to drinking water.

First experiment

Seventeen foxes with known CHV antibody status (14 seronegative, three seropositive) but unknown CHV carrier status were housed in pairs and treated once intramuscularly with 5 mg/kg body weight methylprednisolone acetate (depomedrol, Uphohn).

Second experiment

Five CHV-seropositive foxes (four males, one female) were selected that had survived an intravenous (IV) CHV challenge as described elsewhere (Reubel et al., 2001). All foxes were from among the 17 animals of the first experiment and were treated twice (2 days apart) intramuscularly with 5 mg/kg body weight methylprednisolone 4 mo after the experimental

TABLE 1. Experimental design, animal allocation, canine herpesvirus (CHV) antibody status prior to treatment, and frequency of methylprednisolone acetate (MPA) treatment.

Fox identification	Sex	Experiment 1	Experiment 2	Experiment 3	CHV antibody status before			MPA treatment in		
					Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
70A12	M	MPA (1)	—	—	Neg	—	—	1×	—	—
95F18	M	MPA (1)	—	—	Neg	—	—	1×	—	—
74935	M	MPA (1)	CHV IV[a] MPA (2)	—	Neg	Pos	—	1×	2×	—
B5341	F	MPA (1)	CHV IV MPA (2)	—	Neg	Pos	—	1×	2×	—
C1C1B	F	MPA (1)	CHV IV MPA (2)	—	Neg	Pos	—	1×	2×	—
C4A37	F	MPA (1)	CHV IV MPA (2)	—	Neg	Pos	—	1×	2×	—
D0579	F	MPA (1)	CHV IV MPA (2)	—	Neg	Pos	—	1×	2×	—
16254	M	—	—	CHV PO[b] MPA (3)	—	—	Pos	—	—	5×
26679	M	—	—	CHV PO MPA (3)	—	—	Pos	—	—	5×
61D71	F	—	—	CHV PO MPA (3)	—	—	Pos	—	—	5×
7064E	M	—	—	CHV PO MPA (3)	—	—	Pos	—	—	5×
E667E	F	—	—	CHV PO MPA (3)	—	—	Pos	—	—	5×
F094D	F	MPA (1)	—	CHV PO MPA (3)	Neg	—	Pos	—	—	5×
76F35	M	MPA (1)	—	—	Neg	—	—	1×	—	—
C1B1F	F	MPA (1)	—	—	Neg	—	—	1×	—	—
6576F	M	MPA (1)	—	—	Pos	—	—	1×	—	—
E360C	M	MPA (1)	—	—	Pos	—	—	1×	—	—
F0C07	M	MPA (1)	—	—	Pos	—	—	1×	—	—
24A19	F	—	MPA (2)-in contact	—	—	Neg	—	—	—	—
51A17	M	—	MPA (2)-in contact	—	—	Neg	—	—	—	—
D445E	F	—	MPA (2)-in contact	—	—	Neg	—	—	—	—
72713	F	MPA (1)	MPA (2)-in contact	—	Neg	Neg	—	1×	—	—
C6E22	F	MPA (1)	MPA (2)-in contact	—	Neg	Neg	—	1×	—	—
74208	M	—	—	MPA (3)-in contact	—	—	Neg	—	—	—
62B65	F	—	—	MPA (3)-in contact	—	—	Neg	—	—	—
F0140	F	—	—	MPA (3)-in contact	—	—	Neg	—	—	—
B3724	M	—	—	MPA (3)-in contact	—	—	Neg	—	—	—
23FOB	M	MPA (1)	—	MPA (3)-in contact	Neg	—	Neg	1×	—	—
E4C72	F	MPA (1)	—	MPA (3)-in contact	Neg	—	Neg	1×	—	—

[a] IV = intravenous inoculation.
[b] PO = peroral inoculation.

CHV IV inoculation. They were then paired and housed together with five CHV-seronegative, in-contact control foxes (four males, one female).

Third experiment

Six CHV-seropositive foxes (three males, three females) were selected that had previously been perorally (PO) inoculated with CHV but had not shown clinical signs of disease (Reubel et al., 2001). Eleven months after the experimental CHV inoculation, these foxes were treated 5 times on 5 consecutive days with 5 mg/kg body weight methylprednisolone, paired, and housed together with six CHV-seronegative in-contact control foxes (three males, three females). The experimental groups and details of the foxes used in the experiments (identification, sex, CHV antibody status, and frequency of corticosteroid treatment) are shown in Table 1.

Clinical evaluation

Body temperatures were taken rectally daily from all foxes over a 14-day period post–methylprednisolone treatment (PT). Body weights were measured weekly in all experimental animals over the entire observation period.

Blood collection, hematology, and serology

Heparinized blood (5 ml) was obtained from foxes either by jugular or cephalic vein puncture. In the first experiment samples were collected on days 0, 7, 14, 21, and 28 PT, in the second experiment weekly for up to 12 wk, and in the third experiment for up to 8 wk PT. In the third experiment, fox plasma samples were

also available from a period of 11 mo prior to the methylprednisolone treatments. Blood samples were centrifuged for 30 min at 800 × G, and the plasma was collected and stored at −20 C. In the second experiment, white blood cells (buffy coat cell layers) were also collected for polymerase chain reaction (PCR) assays from five CHV IV–infected and five uninfected control foxes on days 0, 7, 14, 21, 28, and 56 and stored at −20 C.

Total white blood cell (WBC) count and number of lymphocytes, neutrophils, eosinophils, and monocytes were determined over a 3–6-wk period PT using a Diff-Quik stain set (Lab Aids Pty, Narrabeen, Australia) and/or standard hematology procedures (Schalm and Jain, 1986).

Antibodies to CHV were determined in plasma samples using a focus–enzyme linked immunosorbent assay (FELISA) as described elsewhere (Reubel et al., 2001). Briefly, Madin Darby canine kidney (MDCK) cells were grown in 96-well plates, infected with CHV, and fixed with methanol when cytopathic effect (CPE) typical for CHV appeared. Serial dilutions of test plasmas were added, incubated for 60 min at 37 C, and washed with phosphate buffered saline (PBS). Biotinylated goat anti-dog IgG (Kirkegaard and Perry, Gaithersburg, Maryland, USA) was then added and the plates incubated for 30 min at 37 C. After another washing step, streptavidin-conjugated horseradish peroxidase (HRP, Sigma, Castle Hill, New South Wales, Australia) was added and the plates incubated for 30 min at 37 C. To visualize the location of streptavidin-HRP bound to viral antigens, substrate AEC (Sigma) was added, which resulted in a reddish-orange staining of CHV-infected cells. Controls were sera from a CHV-seronegative dog (negative control) and from a CHV IV–infected fox (positive control). A titer of ≥1:10 was considered positive.

Collection of mucosal secretions

Sterile cotton swabs (Sarstedt, Nümbrecht, Germany) were used to collect secretions from eye, nose, and throat (one combined swab) and from vaginal or prepuce secretions (swab two). Prior to sampling, swabs were kept in sterile tubes containing 1.0 ml of sterile PBS. After sampling, the fluid was squeezed out of the cotton, filtered through a 0.22-μm disposable filter (Millipore, Eschborn, Germany), and stored at −70 C. All foxes were sampled daily over a 14-day period PT.

Collection of trigeminal ganglia

The trigeminal ganglion is a preferred site of CHV latency in dogs (Miyoshi et al., 1999) and was therefore selected in this study for PCR analysis. Trigeminal ganglia were collected postmortem from six foxes that had previously been experimentally infected with CHV, either IV or PO. Two foxes (C1C1B, C4A37) were autopsied 26 mo after CHV IV infection; four foxes (16254, 26679, 61D71, 7064E) were autopsied 18 mo after CHV PO inoculation.

Virus isolation

Madin Darby canine kidney cells were grown in 48-well microtiter plates and inoculated in duplicate with 30 μl of each previously filtered swab sample. Inoculated cell cultures were incubated for 24 hr at 37 C and 5% CO_2 and then the media were replaced to minimize possible toxic effects of the inoculum to the cells. Plates were incubated for an additional 5 days and checked daily for CPE. Supernatants of wells with visible CPE were harvested and assayed by PCR to confirm the presence of CHV DNA.

Polymerase chain reaction

Genomic DNA was extracted from selected tissues (trigeminal ganglia and buffy coat cells) by standard phenol/chloroform method (Sambrook et al., 1989). Approximately 500 ng of DNA was used as PCR template. Filtered mucosal secretions were used without further preparation at a volume of 3 μl/PCR assay. A nested PCR assay for detection of the CHV thymidine kinase gene (TK) sequence was used as described elsewhere (Reubel et al., 2001), which predicted PCR products of 508 base pairs (bp) for the first round and 336 bp for the nested round of amplification, respectively. This PCR assay was previously shown to be 100-fold more sensitive in detecting CHV than virus isolation by cell culture (Reubel et al., 2001). The absolute sensitivity limit of CHV DNA detection by PCR was established by assaying limiting dilutions of DNA derived from plasmid pCMH396. This plasmid has a molecular size of 9.4 kb and contains the complete TK gene of CHV (Reubel et al., 2002). Double-distilled water and/or genomic DNA from a known CHV seronegative fox was used as a negative control, positive controls were CHV DNA and/or CHV TK DNA from plasmid pCKMH396. To assess the specificity of the assay, canine adenovirus DNA and vaccinia virus DNA were used as templates in selected assays.

RESULTS

Clinical signs, hematology, and serology

Clinical signs of CHV-induced disease or immunosuppression such as opportu-

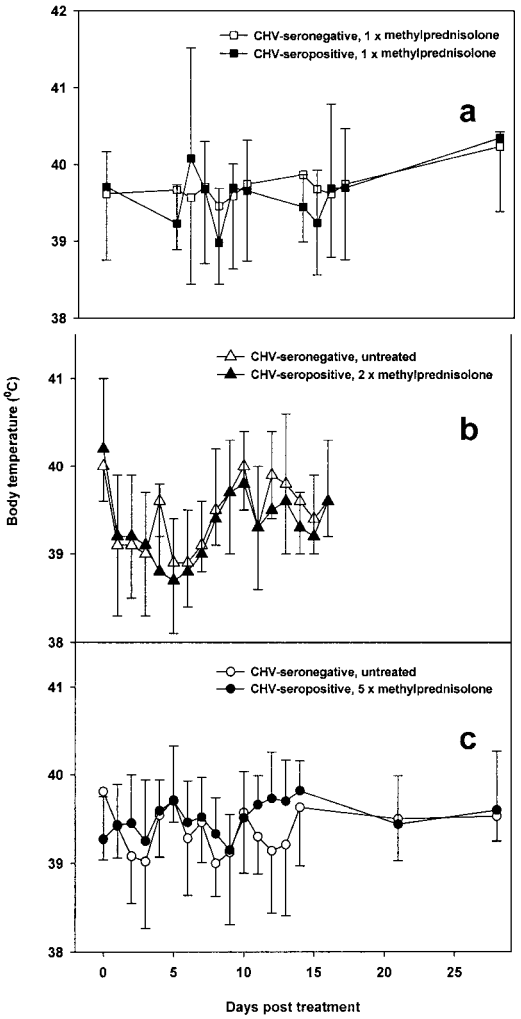

FIGURE 1. Body temperatures (a) of 17 foxes with unknown canine herpesvirus (CHV) carrier status (14 CHV seronegative [□], three CHV seropositive [■], after one treatment with methylprednisolone), (b) of five untreated, CHV-seronegative, in-contact control foxes [△] compared with five CHV intravenously infected foxes [▲] that were twice treated with methylprednisolone, and (c) of six untreated, CHV-seronegative, in-contact control foxes [○] compared with six CHV perorally infected foxes [●] that were treated five times with methylprednisolone, (mean±standard deviation).

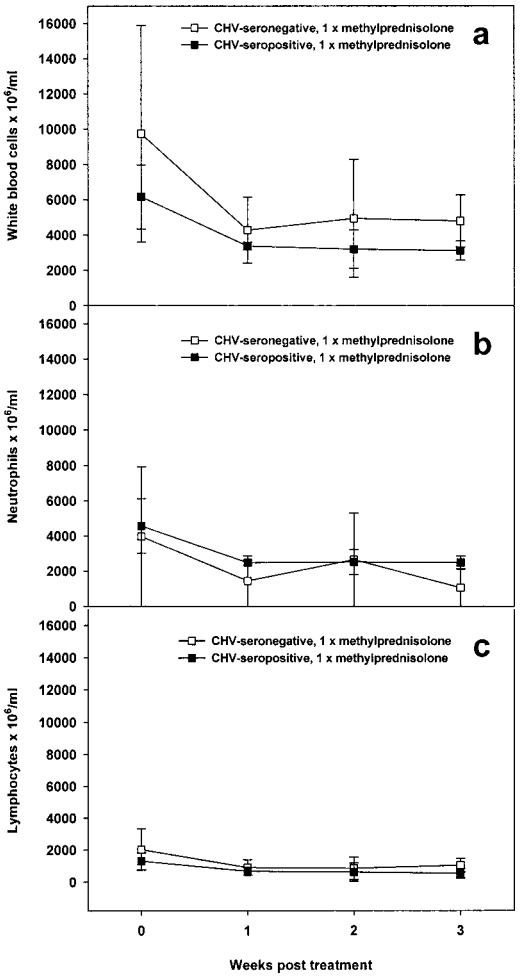

FIGURE 2. (a) Total white blood cells, (b) number of neutrophils, and (c) number of lymphocytes of 17 foxes with unknown canine herpesvirus (CHV) carrier status (14 CHV seronegative [□], three CHV-seropositive [■], all treated once with methylprednisolone), (mean±standard deviation).

nistic bacterial infections were not observed in any of the foxes following methylprednisolone treatment. Body temperatures did not change substantially after treatment, either in foxes with unknown CHV carrier status (Fig. 1a) or in previously experimentally CHV IV– or CHV PO–infected foxes (Fig. 1b, c). Body weights also did not change following treatment in any of the three experiments (data not shown).

In the first experiment (Fig. 2), all 17 foxes received one treatment with methylprednisolone. Prior to treatment, the WBC count varied considerably among individual foxes regardless of their CHV antibody status (Fig. 2a). The very high WBC count in two foxes contributed to the high standard deviation for the first data point

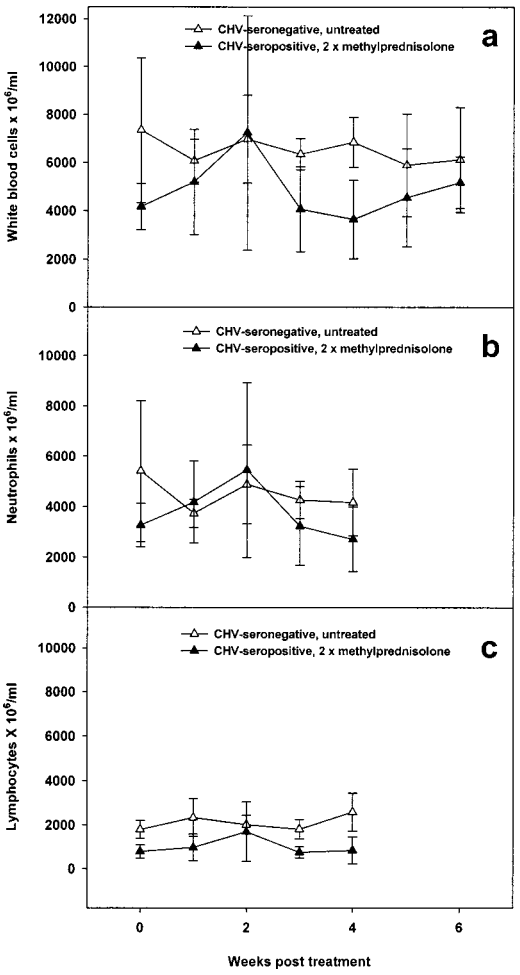

FIGURE 3. (a) Total white blood cells, (b) number of neutrophils, and (c) number of lymphocytes of five untreated, canine herpesvirus (CHV)-seronegative, in-contact control foxes [△] compared with five CHV intravenously infected foxes [▲] that were twice treated with methylprednisolone (mean±standard deviation).

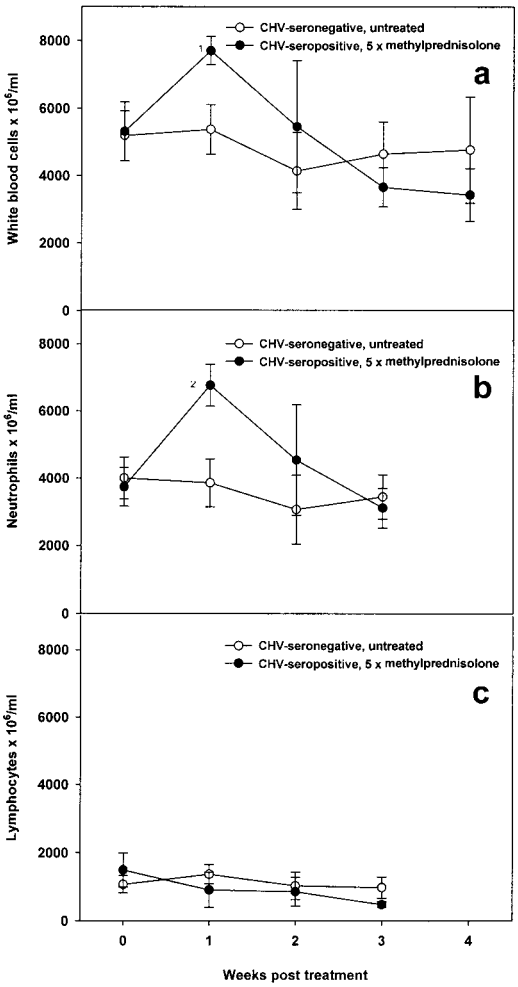

FIGURE 4. (a) Total white blood cells, (b) number of neutrophils, and (c) number of lymphocytes of six untreated, canine herpesvirus (CHV)-seronegative, in-contact control foxes [○] compared with six CHV perorally infected foxes [●] that were treated five times with methylprednisolone (mean±standard deviation). [1]Paired Student's *t*-test $P=0.0033$; [2]Paired Student's *t*-test $P=0.0020$.

and was most likely caused by bacterially infected skin lesions from which these individuals suffered at that time. However, during 3 wk PT mean numbers of neutrophils or lymphocytes did not change substantially and there was also no major difference between seronegative and seropositive foxes (Fig. 2b, c).

In the second experiment (Fig. 3), CHV IV–infected foxes were twice treated with methylprednisolone. There was no major difference in the WBC counts of those foxes compared to those of uninfected, untreated control foxes (Fig. 3a). No change in total WBC counts attributable to the treatment was observed. The neutrophil and lymphocyte numbers remained at the same level in both CHV-seropositive and -seronegative foxes during the entire observation period, although there was considerable variation between individuals (Fig. 3b, c).

In the third experiment (Fig. 4), CHV

PO–infected foxes were given methylprednisolone treatment on 5 consecutive days. There was considerable fluctuation in WBC counts (Fig. 4a) and neutrophil numbers (Fig 4b) over the course of the experiment. However, with the exception of week 1 PT, the data were not substantially different from those obtained from untreated controls. There was no major change in lymphocyte numbers in seropositive, treated foxes and seronegative, untreated controls (Fig. 4c).

In all three experiments there were no major changes in eosinophil or monocytes numbers after treatments (data not shown).

Virus isolation

Infectious CHV was not isolated from either oral/nasal/ocular or vaginal/preputial secretions of any of the foxes after methylprednisolone treatments.

Polymerase chain reaction

Using CHV DNA or CHV TK plasmid pCMH396 DNA as a template, first-round and nested-round PCR produced DNA fragments of the predicted size of 508 and 336 bp, respectively. Double-distilled water, genomic DNA from a CHV-seronegative foxes, canine adenovirus DNA, and vaccinia virus DNA used as control templates produced no visual DNA fragments (data not shown). The sensitivity of the PCR assays allowed routine estimated detection of 10–100 copies of CHV TK DNA derived from plasmid pCMH396. All mucosal secretions taken from foxes during the course of the three experiments tested PCR negative for CHV DNA. All buffy coat cell samples taken weekly over a 6-wk period from experimentally CHV IV–infected foxes that were treated twice with corticosteroids were also PCR negative, as were those from uninfected in-contact controls. The six trigeminal ganglia taken postmortem from experimentally CHV-infected foxes (either IV or PO) were positive for CHV DNA (Fig. 5).

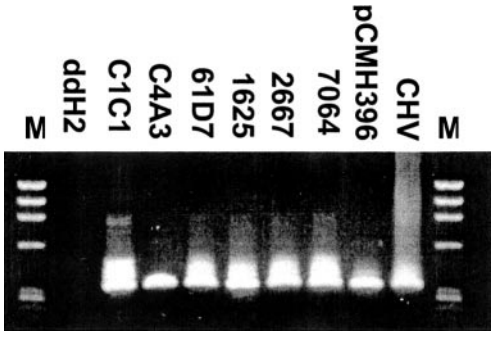

FIGURE 5. Polymerase chain reaction detection of canine herpesvirus (CHV) DNA in trigeminal ganglia of six experimentally CHV-infected foxes (identification numbers indicated). M=molecular size marker; ddH2O, double-distilled water=negative control; pCMH396 DNA and CHV DNA=positive controls.

Serology

In the first experiment (Fig. 6a), three of 17 foxes had anti-CHV antibodies prior to the corticosteroid treatment and 14 of 17 were seronegative. None of the CHV-seronegative foxes seroconverted PT. In the CHV-seropositive group, the small fox number (n=3) with very high individual variance in anti-CHV antibody titers caused a high standard deviation at the first two data points. However, a rise in anti-CHV antibody titers following the treatment was not observed.

In the second experiment (Fig. 6b), the anti-CHV antibody titers of the experimentally CHV IV–infected foxes ranged from 1:1,000 to 1:8,000 prior to the double corticosteroid treatment. The mean anti-CHV antibody titer fluctuated during the course of the experiment but did not substantially increase following the treatment. All foxes in the untreated, in-contact control group were CHV seronegative prior to the experiment and remained negative during the observation period (5 wk).

In the third experiment (Fig. 6c), anti-CHV antibody titers were measured during a period of more than a year after the initial peroral CHV inoculation and for 8 wk following methylprednisolone treatment. Two weeks after the initial infection, the six foxes in this experimental group re-

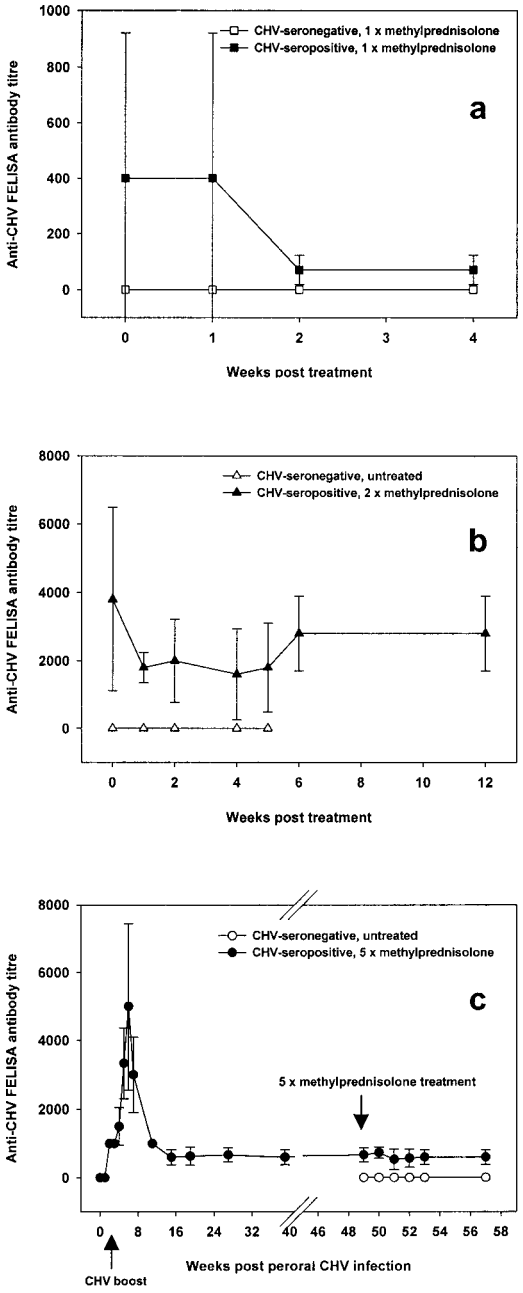

FIGURE 6. Anti–canine herpesvirus (CHV) focus–enzyme-linked immunosorbent assay (FELISA) antibody titers of (a) 17 foxes with unknown CHV carrier status (14 CHV seronegative [□], three CHV seropositive [■], after one treatment with methylprednisolone), (b) of five untreated, CHV-seronegative, in-contact control foxes [△] compared with five CHV intravenously infected foxes [▲] that were twice treated with methylprednisolone, and (c) of six untreated, CHV-seronegative, in-contact control foxes [○] compared with six CHV perorally infected foxes [●] that were treated five times with methylprednisolone (mean±standard deviation). In (c), the second CHV inoculation (boost infection) is indicated by an arrow.

ceived a CHV boost that triggered a sharp rise in anti-CHV antibodies lasting from week 6 to week 10. These results have been previously reported (Reubel et al., 2001). About 16 wk after the initial infection, anti-CHV antibodies returned to levels of around 1:1,000, where they remained until the end of the observation period (week 58). Five treatments with methylprednisolone on 5 consecutive days had no influence on the mean CHV antibody pattern of those foxes. Six untreated, CHV-seronegative control foxes that were housed together with the infected and treated foxes remained seronegative during the course of the experiment.

DISCUSSION

The aim of the present study was to explore whether glucocorticoid treatment of latently CHV-infected red foxes would trigger reactivation and shedding of infectious CHV. We used the drug methylprednisolone acetate, which causes immunosuppression in cats and dogs, which has previously been applied to induce shedding of latent infectious feline herpesvirus (FHV) and CHV from experimentally infected animals (Kruger et al., 1990; Okuda et al., 1993a, b; Reubel et al., 1993; Hickman et al., 1994; Maggs et al., 2003). After acute infection, both viruses are known to become latent in a variety of tissues of those species, with the trigeminal ganglion a consistent site of latency (Okuda et al., 1993b; Reubel et al., 1993; Burr et al., 1996; Weigler et al., 1997; Miyoshi et al., 1999). We have reported that in red foxes the trigeminal ganglia were frequently PCR positive for CHV DNA after experimental CHV infection (Reubel et al., 2001). However, no reactivation and/or shedding of CHV from this noninfectious state has been described for foxes.

The most important finding of this study was that none of the CHV-seropositive foxes shed infectious CHV following methylprednisolone treatment. As reported elsewhere, five of five CHV IV–infected foxes and four of six CHV PO–infected foxes shed infectious CHV or CHV DNA into mucosal secretions (Reubel et al., 2001). Moreover, the trigeminal ganglia from six of those foxes tested positive for CHV DNA by PCR. This is clear evidence that the experimentally infected foxes were CHV carriers at the time of methylprednisolone treatment. However, all 82 samples taken after treatment from oral, nasal, ocular, and genital secretions of these foxes contained neither infectious CHV or CHV DNA. This is in contrast to the situation in latently CHV-infected dogs or FHV-infected cats where virus shedding into mucosal secretions can readily be induced by corticosteroid treatment (Okuda et al., 1993a, b; Reubel et al., 1993; Hickman et al., 1994). The method of virus isolation used in the present study is well suited to detect infectious CHV in mucosal secretions of foxes (Reubel et al., 2001) and therefore does not appear to be the reason for the failure to isolate virus in cell culture. Furthermore, the sensitivity of the PCR assay used in this study was very high and routinely detected between 10 and 100 viral copies, and so the applied diagnostic methods were sensitive enough to detect reactivated virus if it occurred. Canine herpesvirus was not isolated from uninfected, untreated in-contact control foxes. This is another important result because these foxes were housed together with CHV-infected individuals for an extended period of time (up to 8 mo). If infected foxes shed the virus at any stage during the experiments, even if undetected by laboratory methods, it is likely that it would have been passed on to seronegative in-contact foxes. However, all of the control foxes remained CHV seronegative during the entire course of the study (up to 8 mo).

These virologic findings are supported by serologic results obtained for the CHV-seropositive foxes. In CHV-infected dogs, the anti-CHV antibody titer reportedly increases significantly after corticosteroid treatment, which can be attributed to an increased exposure to viral antigens (Okuda et al., 1993a). A similar rapid increase in anti-CHV antibodies was observed also in foxes after a peroral CHV boost (Reubel et al., 2001). However, none of the methylprednisolone treatments in this study led to major increases in anti-CHV antibodies.

The frequency and/or dose of methylprednisolone treatments did not appear to influence the immune defenses of foxes in this study and, indirectly, the rate of virus isolation or PCR positivity. Comparable amounts of prednisolone (approximately 5 mg/kg body weight) given to dogs readily induced CHV shedding (Okuda et al., 1993a, b). Methylprednisolone acetate has a prolonged immunosuppressive effect on dogs (up to 6 wk) and was expected to have an even more profound effect on immune cells than prednisolone. In a previous study, less than half the dose of prednisolone (2 mg/kg body weight) was administered to red foxes to alter the distribution of rabies vaccine virus, but the effect of the treatment was inconclusive (Ciuchini et al., 1986).

Reasons for the failure to induce shedding from known CHV-positive foxes by methylprednisolone treatment are unclear. Although prednisolone derivatives have been successfully used in the past to induce herpesvirus shedding in a variety of latently infected species, the effects of corticosteroid treatment on the immune system of foxes are unknown. It has been reported that even large doses of prednisolone did not induce virus shedding in known CHV-positive dogs (Okuda et al., 1993a). Clinically and hematologically, there were no obvious signs in the present study that methylprednisolone treatment caused immunosuppression in red foxes. Clinical parameters such as body temperature and body weight remained unchanged following treatment. Also hema-

tologic data were relatively unaffected by treatment and not substantially different from uninfected and treated animals (experiment one) or uninfected and untreated controls (experiments two and three). In a similar study in dogs, prednisolone treatment resulted in an increase of leukocytes 7 days following corticosteroid treatment and a sharp decrease of lymphocyte numbers 1 wk after treatment (Okuda et al., 1993a). A significant increase of total white blood cells was seen also in this study 1 wk after five methylprednisolone treatments (experiment three). However, the lymphocyte numbers remained unchanged.

There is no evidence from this study that CHV is readily reactivated from CHV-seropositive carriers and passed on to seronegative in-contact foxes, although they stayed in close proximity over an extended period of time. This could explain why the CHV seroprevalence in European red foxes in Australia is very low. Based on results presented here it appears that bait-vaccinated foxes will not shed sufficient CHV with their mucosal secretions into the environment to infect other, naive foxes. However, similar studies with any recombinant vaccine virus will still need to be conducted to confirm this finding.

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