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Characterization of Dexamethasone-Induced Reactivation of Latent Bovine Herpesvirus 1

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Synchronous reactivation of bovine herpesvirus type 1 in all latently infected rabbits was achieved following a single intravenous dose of dexamethasone. Reactivated latent virus was first present in ocular secretions between 48 and 72 h post-dexamethasone treatment (PT). Cell-free infectious virus, viral-antigen-containing neurons, and pathologic changes were detectable in trigeminal ganglia (TG) by 48 h PT. A shift from the viral transcriptional pattern characteristic of the latent state (latency-related RNA [LR RNA]) to one typical of that seen during acute infection was detected in a small number of neurons in latently infected TG between 15 and 18 h PT, with viral DNA first detectable by in situ hybridization at 18 to 21 h PT. The number of LR RNA-containing neurons in latently infected TG decreased significantly at 24 and 48 h PT but returned to near-normal levels by 72 h PT. Correlation of this decrease with viral reactivation suggests that altered regulation of LR RNA transcription is a significant event in the process of viral reactivation.

Bovine herpes virus type 1 (BHV-1), a member of the alphaherpesvirus group, is a significant pathogen of cattle responsible for a variety of disease conditions, including rhinotracheitis, conjunctivitis, genital infections, meningoencephalitis, and fatal systemic infection (10). BHV-1 establishes latent infections in neurons of sensory and autonomic nerve ganglia and is reactivatable. In all likelihood, it is this viral characteristic alone that is responsible for perpetuation and transmission of infection in cattle (2, 7, 8, 13, 14, 22). A rabbit latency model that is consistent with the observations made for latently infected cattle has been developed and used subsequently to examine the molecular basis of BHV-1 latency (17).

As with other alphaherpesviruses, the molecular mechanisms that underlie BHV-1 latency and reactivation are poorly understood. Restricted transcription of the BHV-1 genome in latency-infected neurons in the natural host and in the rabbit latency model has been described. BHV-1 latency-related RNA (LR RNA), present in neurons of trigeminal ganglia (TG) from latently infected rabbits and cattle, is predominantly nuclear and approximately 0.77 to 1.16 kb in size and maps to a region of the viral genome that overlaps an uncharacterized immediate-early (IE) gene present on the complementary strand (12, 18). The significance of this transcription in latency and reactivation is unknown.

One particularly intriguing feature of BHV-1 infection is that virus can be predictably reactivated from virtually all latently infected animals following the administration of dexamethasone, a synthetic glucocorticoid (5, 11, 17, 21). Glucocorticoid-mediated BHV-1 reactivation is likely a significant mechanism underlying the survival of BHV-1 in nature; naturally occurring stressful conditions may lead to increases in endogenous corticosteroids and subsequent viral reactivation and shedding in latently infected animals (5). Glucocorticoids are known regulators of both cellular and viral gene expression and, in addition, they are known to be potent immunosuppressive agents in vivo (20, 25). The manner by which dexamethasone induces BHV-1 reactivation is unknown; however, it appears that the immunosuppression associated with treatment is not itself directly responsible (5).

In this study, we have used the rabbit latency model to examine early ganglionic events surrounding dexamethasone-induced BHV-1 reactivation.

MATERIALS AND METHODS

Cells and virus. Bovine turbinate or bovine lung cells were used for these experiments and maintained as described previously (17). The Cooper strain of BHV-1, supplied by the National Veterinary Services Laboratory, Ames, Iowa, was used for animal inoculation at passage 6.

Animal infections and dexamethasone reactivation. New Zealand White rabbits were lightly anesthetized with methoxyflurane and inoculated in the right and left conjunctival sacs with 10^7 PFU of BHV-1 as described previously (17). Latently infected rabbits (2 to 4 months postinfection) were treated with a single intravenous dose of dexamethasone (2.8 mg/kg of body weight) and killed at various times posttreatment (PT). Ocular swabs from latently infected rabbits were collected and cultured for 2 consecutive days before dexamethasone treatment to verify that spontaneous reactivation was not occurring (17). Acutely infected TG were obtained from rabbits 4 days postinfection.

Virus isolation, histopathology, and immunofluorescence. Ocular swab samples were handled as described previously (17). Ganglionic homogenates (10% in minimal essential medium) prepared with Ten Broeck tissue grinders were assayed with bovine cells. Ganglia for explant culture were cut into 1- to 5-mm pieces and cultured in minimal essential medium supplemented with 20% fetal bovine serum. Cultures were maintained for 30 to 45 days before being discarded as negative. Tissue samples for histopathology were fixed in 10% neutral buffered formalin and processed by standard paraffin procedures. Sections were stained with hematoxylin and eosin. Tissue samples for immunofluorescence were rapidly frozen; sections of 5 to 7 μm were cut and fixed in room temperature acetone for 5 min. Indirect immunofluorescence was performed with a hyperimmune bovine anti-BHV-1 serum and a fluorescein-labelled goat

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anti-bovine immunoglobulin G conjugate. Controls included uninfected and BHV-1-infected bovine lung cells, as well as sections from uninfected TG.

In situ hybridization. In situ hybridization was performed essentially as described previously (3, 18, 23). Animals were lightly anesthetized and killed by barbiturate overdose. TG were dissected and then immersed in periodate-lysine-paraformaldehyde fixative for 24 h at 4°C before being embedded in paraffin for sectioning. Tissue sections were deparaffinized with xylene, rehydrated in graded ethanols, pretreated with 0.2 N HCl for 20 min, and then treated with proteinase K (1 mg/ml) in 10 mM Tris (pH 7.4)–2 mM CaCl for 15 min at 37°C. Sections probed for viral DNA were rinsed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), further treated with DNase-free RNase A (100 μg/ml in 2× SSC) for 30 min at 37°C, and refixed in 5% paraformaldehyde for 30 min. Prior to hybridization, DNA was denatured by being heated to 65°C in deionized formamide in 0.1× SSC for 15 min and then quenched in ice-cold 0.1× SSC. The sections were then probed with various ^{3}H-labelled BHV-1 DNA fragments (~10^5 cpm) in a hybridization solution containing 2× SSC, 45% formamide, 10% dextran sulfate, 10 mM EDTA, 1× Denhardt solution (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), and 0.25 mg of rabbit brain total nucleic acids per ml. After hybridization, sections were washed in 2× SSC–45% formamide–10 mM Tris (pH 7.4)–1 mM EDTA for 1 day at room temperature, coated with Kodak NTB-2 emulsion, exposed for 14 to 21 days at 4°C, and developed and stained with hematoxylin and eosin as previously described (24). Sections were examined and scored in a masked fashion.

Quantitative in situ hybridization: experimental design and statistical analysis. Uniform stratified samples of ganglionic tissue were examined; ganglia were completely serially sectioned, and alternate sections from throughout each ganglion were probed for LR RNA (with a 955-bp *Pst*I-*Sal*I fragment from the LR transcriptionally active region [see Fig. 2]) or viral DNA (with *Hin*dIII fragment K). Quantitative studies of LR RNA-containing neurons were accomplished by using a randomized complete-block analysis of variance design. Quantitative comparisons over time of LR RNA- and viral-DNA-containing cells were analyzed with a randomized, complete-block, repeated-measures analysis of variance. The statistical design of these experiments was based on preliminary studies that estimated the proportion of latently infected cells (data not shown). In all cases, the design would detect a 40% difference at an alpha level of 0.05 and a beta level of 0.80. It should be noted that this statistical design would not be able to reliably detect statistically significant differences of less than 40%.

RESULTS

Dexamethasone-induced BHV-1 reactivation. Dexamethasone induces efficient reactivation of latent BHV-1 in both rabbits and cattle (5, 11, 17, 21). In previous experiments, multiple-dose regimens in which the drug was administered intramuscularly over several days were used. Here, we examined whether a single dose of dexamethasone (2.8 mg/kg) administered intravenously would be (i) sufficient for inducing efficient BHV-1 reactivation and (ii) capable of providing relatively synchronous viral reactivation in latently infected TG. After a single intravenous dose of dexamethasone, ocular swabs were obtained from latently infected rabbits at various times PT to test for the presence of infectious virus at the original inoculation site. Reactivated virus was recovered from ocular swabs of approximately 80% of the animals examined at 48 h PT and from 100% of the animals at 72 h PT (Table 1). Untreated latently infected control animals showed no evidence of viral reactivation and shedding.

TABLE 1. Ocular shedding of BHV-1 following dexamethasone-induced viral reactivation in latently infected rabbits[a]

Time PT (h)	No. of virus-positive rabbits/total no. examined			
	Expt 1	Expt 2	Expt 3	Untreated controls
0	0/5	0/5	0/9	0/5
24	0/5		0/9	0/5
48	3/5		8/9	0/5
72	5/5	5/5	9/9	0/5
96	5/5			0/5
120				0/5

[a] 2.8 mg of dexamethasone per kg was administered intravenously at 0 h.

Virologic and histopathologic examination of latently infected TG during dexamethasone-induced viral reactivation. Latently infected TG were evaluated for the presence of cell-free infectious virus and viral-antigen-containing cells at various times PT. In contrast to results seen with untreated latently infected TG, BHV-1 was recoverable from ganglionic tissue homogenates 48 h PT, with BHV-1-specific fluorescent cells detectable at both 48 and 72 h PT (Table 2). Fluorescence was observed in a small number of neurons and occasionally in unidentified small cell types (Fig. 1C).

Pathologic changes in TG were observed in one of three animals examined at 24 h PT and in all animals at 48 and 72 h PT (n = 6). Lesions consisted of focal interstitial mononuclear cell accumulations with focal neuronal degeneration, neuronal necrosis, and neuronophagia. With in situ nucleic acid hybridization, degenerative neuronal changes were observed in some neurons containing BHV-1 DNA (Fig. 1D).

These results indicate that during dexamethasone-induced BHV-1 reactivation, infectious BHV-1 and viral-antigen-containing cells can first be detected in latently infected TG some time between 24 and 48 h PT and that pathologic changes consistent with focal neuronal cell death occur in reactivating ganglia. Temporally, these ganglionic changes precede, or are concurrent with, the appearance of reactivated virus at the ocular site.

Transcription from the latent BHV-1 genome during dexamethasone-induced reactivation. To investigate BHV-1 gene transcription and viral DNA synthesis during induced reactivation, in situ nucleic acid hybridization, with individual

TABLE 2. Virologic and histopathologic examination of latently infected rabbit TG during dexamethasone-induced viral reactivation

Time PT (h)	No. of virus-positive rabbits/total as determined by:				
	Virus isolation			Viral-antigen detection[a]	Pathologic changes
	Ocular swab	Explant culture	Tissue homogenate		
0	0/5	5/5	0/5	0/7	1/3
24	0/3	3/3	0/3	0/3	1/3
48	0/3	3/3	2/3	2/3	3/3
72	3/3	3/3	0/3	2/3	3/3

[a] Detection with indirect fluorescence procedures.

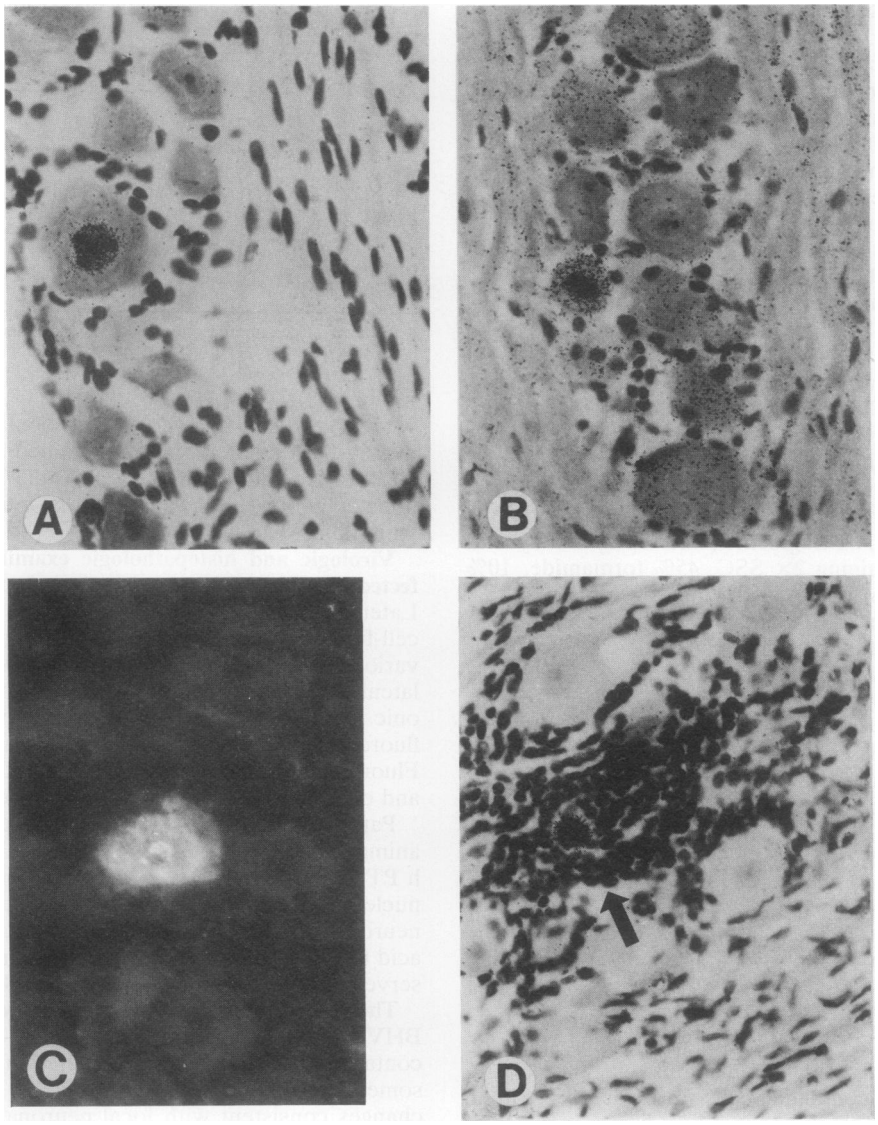

FIG. 1. Detection of BHV-1 antigens and nucleic acid in latently infected rabbit TG during dexamethasone-induced viral reactivation. (A) Section of control latently infected rabbit TG hybridized with a probe (*Pst*I-*Sal*I fragment, nucleotides (81 to 1936) (Fig. 2) to detect LR RNA. (B) Section of TG at 21 h PT hybridized with *Hin*dIII fragment K (Fig. 2) to detect viral RNA. A single neuron containing nuclear and cytoplasmic RNA transcribed from this region is shown. (C) BHV-1 specific nuclear and cytoplasmic fluorescence is present in a single neuron at 72 h PT. (D) Section of TG at 30 h PT probed for BHV-1 DNA. Note the accumulation of inflammatory cells and the developing neuronophagic nodule around a neuron containing BHV-1 DNA (arrow).

cloned *Hin*dIII fragments spanning the BHV-1 genome as probes (Fig. 2), was performed on sections of TG taken from latently infected animals at various times following dexamethasone treatment. Results from these experiments are shown in Table 3. From 0 to 15 h PT, the hybridization pattern seen for the ganglia was similar to that observed for untreated latently infected ganglia; transcription was restricted to *Hin*dIII fragment D, the genomic region encoding the BHV-1 LR RNA(s) (12, 18). Between 15 and 18 h PT, transcription from other regions of the genome was observed in neurons, and by 21 to 24 h PT, probes from throughout the genome hybridized to most ganglion tissue samples (Table 3 and Fig. 1B).

Viral DNA was first detected by in situ hybridization in neurons of reactivating ganglia between 18 and 21 h PT. As described previously, the DNA hybridization signal was localized to the cell nucleus (19). In some instances, viral-DNA-containing cells present in inflammatory foci showed evidence of necrotic changes, including pyknotic nuclei, pale-staining eosinophilic cytoplasm, and irregular and fragmented cell membrane borders. Thus, by 21 h PT, the hybridization pattern seen with reactivating ganglia was qualitatively indistinguishable from that seen with ganglia of acutely infected animals (Table 3) (18). Quantitatively, however, the number of hybridizing neurons in reactivating ganglia was appreciably lower.

These results indicate that a 15- to 18-h lag period precedes extensive latent genome transcription following dexamethasone treatment. Once additional viral transcription begins, it is extensive, spans the entire genome, and is

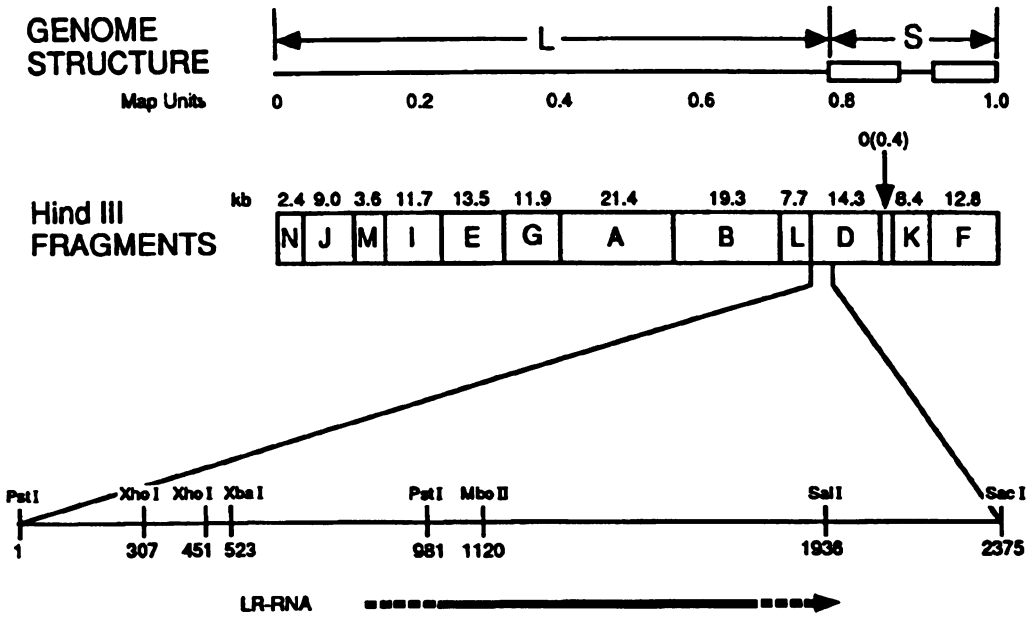

FIG. 2. BHV-1 DNA (Cooper strain). This linear double-stranded molecule of approximately 136 kb consists of two unique sequence regions, the smaller of which is bounded by inverted repeat sequences. Approximate map locations of the LR RNA (5′ to 3′) in rabbit and bovine TG are shown under an expanded map of the BHV-1 LR transcriptionally active region (12). The 5′ end of the RNA is located within nucleotides 773 to 981, with the 3′ end located between nucleotides 1753 and 1936.

closely coupled to the appearance of viral DNA detectable by in situ hybridization.

Frequency of LR RNA-containing neurons during dexamethasone-induced viral reactivation. In order to determine whether the number of LR RNA-containing neurons in latently infected TG fluctuates during or following dexamethasone-induced reactivation, quantitative in situ hybridization studies were performed. In these experiments, uniform stratified samples of TG were obtained from untreated and dexamethasone-treated animals at various times PT, and then alternate sections throughout the ganglion were probed for either LR RNA or viral DNA. Following hybridization, ganglionic sections, representing between 6,000 and 56,000 neurons per animal, were scored in a masked fashion for hybridization with the probes.

The mean frequency of LR RNA-containing neurons in ganglia from untreated latently infected animals was 0.085%, with individual animal values ranging from 0.05 to 0.22% (Table 4). Viral-DNA-containing neurons were not detected in any ganglionic sections from this untreated group of animals. At 24 h PT, the number of LR RNA-containing neurons was significantly decreased (by 45% ± 19% [P = 0.028] from untreated-control values [Table 4]). At 48 h PT, the number had fallen 59% ± 18% (P = 0.003). Interestingly, the number of LR RNA-hybridizing cells returned to near-normal untreated-control values by 72 h PT (+3% ± 22%; P = 0.89).

Viral-DNA-containing cells were detectable in small numbers (0.01 to 0.018%) at all times examined PT. At their highest mean frequency of 0.018% (48 h PT), they represented approximately 21% of the number of LR RNA-containing neurons present in untreated ganglia.

TABLE 3. BHV-1 RNA and DNA synthesis in rabbit TG during dexamethasone-induced reactivation as detected by in situ hybridization

Rabbit group and time PT (h)	No. of hybridization-positive rabbits/total no. tested									
	RNA synthesis[a]									DNA synthesis
	J	I	E	G	A	B	L	D	K	
Treated										
1	0/3	0/3	0/3	0/3	0/3	0/3	0/1	2/3	0/3	0/3
6	0/3	0/2	0/3	0/2	0/3	0/2	0/3	2/3	0/2	0/3
12	0/6	0/5	0/6	0/3	0/6	0/5	0/5	6/6	0/5	0/6
15	0/4	0/5	0/5	0/5	0/5	0/5	0/6	5/5	0/5	0/3
18	0/4	2/5	1/5	3/5	4/5	1/5	1/3	5/5	1/4	1/3
21	4/5	2/5	3/5	3/5	3/4	1/5	1/4	5/5	3/4	2/3
24	1/2	3/3	3/3	3/3	2/3	3/4	3/3	3/3	3/3	5/9
72										4/5
Uninfected (control)	0/4	0/4	0/4	0/4	0/4	0/3	0/4	0/4	0/3	0/5
Acutely infected[b]	2/3	3/3	1/2	3/4	2/3	2/4	2/2	2/3	2/2	3/3

[a] As determined by using the indicated *Hin*dIII fragments as probes.
[b] Tested 4 days postinoculation.

DISCUSSION

These data taken together, including ganglionic changes and isolation of reactivated virus from the ocular site, indicate that a single intravenous dose of dexamethasone is sufficient for efficient and synchronous reactivation of latent BHV-1 in the rabbit model. The rapid changes in TG observed after a single dose of dexamethasone suggest a direct effect of dexamethasone on the virus-neuron interaction and thus provide additional evidence supporting a nonimmunological mechanism of action for corticosteroids in viral reactivation (5). The observation that ganglionic changes precede the appearance of virus at the ocular site directly supports a pathogenesis sequence for recurrent infection after corticosteroid treatment in which ganglionic latency is biologically relevant and reactivated virus is

TABLE 4. Frequencies of LR-RNA- and viral DNA-containing neurons in latently infected TG after dexamethasone treatment

Time PT (h) and expt no.	Animal no.	No. of neurons hybridizing/total no. tested	
		LR RNA containing	DNA containing
0			
1	A-129	44/19,922	0/28,460
	A-130	24/34,173	0/37,970
	A-131	16/14,466	0/7,233
	A-133	12/8,340	0/11,676
2	A-148	48/65,662	0/57,818
	A-185	23/33,190	0/27,440
	A-186	21/41,344	0/41,288
	A-187	44/52,216	0/52,930
	A-188	29/37,203	0/45,529
Total (%)		261/306,516 (0.085)	0/310,344 (0)
24			
1	A-119	12/17,760	3/20,720
	A-120	3/6,070	0/12,140
	A-128	19/18,755	4/20,840
2	A-166	26/52,150	3/37,170
	A-179	19/40,386	0/47,778
	A-180	5/28,328	5/41,955
	A-182	20/43,967	8/42,741
Total (%)		104/207,416 (0.05)	23/223,344 (0.01)
48			
1	A-114	21/34,290	5/34,290
	A-116	17/32,790	10/32,790
	A-123	8/21,410	12/21,410
	A-125	8/17,941	7/23,067
	A-126	12/28,782	8/31,980
2	A-174	19/44,160	1/42,123
	A-175	16/46,293	8/49,049
	A-176	4/44,942	5/32,994
	A-177	20/32,497	0/37,848
	A-178	12/24,352	5/30,223
Total (%)		137/327,457 (0.042)	61/335,774 (0.018)
72			
1	A-118	37/39,600	10/39,600
	A-127	42/18,726	9/18,726
2	A-171	8/32,656	4/52,934
	A-172	50/40,038	6/41,154
	A-173	23/59,498	4/56,311
Total (%)		160/190,518 (0.084)	33/208,125 (0.016)

translocated back to the ocular site. A combination of observations for reactivating ganglia, including extensive viral transcriptional changes, evidence of viral DNA determined by in situ hybridization, the presence of infectious virus and viral-antigen-containing cells, and neuronal pathology, suggests that dexamethasone-induced BHV-1 reactivation results in lytic viral replication in reactivating neurons that causes cell death. Similar histopathologic changes in TG, including ganglionitis, degeneration of neurons, and neuronophagia, have been observed in latently infected calves and rabbits as early as 3 days post-dexamethasone treatment (4, 6, 15, 16).

Extensive changes in latent viral transcription were first seen 15 to 18 h PT. These data cannot, however, exclude the possibility that even earlier transcription from *Hin*dIII fragment D—a region coding for the LR RNA and other genes, including IE genes (12, 24)—might be occurring. In either case, major changes in viral gene expression occur only after a 15- to 18-h lag period. A similar, although somewhat shorter, lag period has been described for α_1-acid glycoprotein gene induction in rat liver following dexamethasone treatment. In that case, accumulation of functional α_1-acid glycoprotein mRNA is first detectable at 4 h PT and continues to increase through 48 h PT (1). The kinetics of dexamethasone induction of α_1-acid glycoprotein mRNA in rat hepatoma cells in culture show detectable levels of mRNA present at 2 h PT, with maximal induction occurring 12 to 16 h PT (1). The lag period observed during BHV-1 reactivation suggests that extensive changes in viral transcription may be secondary to some primary event, perhaps involving a direct effect of dexamethasone on the neuron itself. During viral reactivation, the reappearance of viral DNA in neurons which is detectable by in situ hybridization is closely coupled to extensive transcription of the latent viral genome. BHV-1 viral DNA, like latent DNA of other alphaherpesviruses, is present in latently infected ganglia but is not detectable by in situ hybridization (Tables 3 and 4) (19, 23). The reason for these failures with what should be sufficiently sensitive in situ hybridization procedures is unknown. Reappearance of viral-DNA-containing neurons during reactivation is most likely due to de novo DNA synthesis that leads to levels of viral DNA comparable to those seen in productively infected neurons. However, the possibility that dexamethasone may alter those conditions responsible for failure to detect latent viral DNA (i.e., induce alterations in the physical structure of the genome or the degree of association with protein) cannot be formally excluded.

At the level of analysis possible from these data, viral reactivation does not appear to be an obviously aberrant process, but rather it resembles the sequence of events observed during acute replication of BHV-1 in TG. In both cases, extensive viral gene expression is closely coupled to viral DNA synthesis, the presence of viral antigens in involved cells, and the production of cell-free infectious progeny (19).

Results from both qualitative and quantitative in situ hybridization studies demonstrate that a small percentage of all LR RNA-containing cells show evidence of altered viral transcription or viral DNA synthesis during dexamethasone-induced reactivation. At most, viral-DNA-containing neurons present at 48 h PT (Table 4) represent approximately 20% of all LR RNA-containing cells present prior to dexamethasone treatment. Thus, it appears that while dexamethasone is capable of inducing viral reactivation in a subset of latently infected cells, it alone is not sufficient to induce reactivation of all latently infected cells and other cellular or viral factors are involved. Data from both bovine and rabbit latency models, in which multiple dexamethasone-induced reactivations of latent virus in the same animal have been achieved, support this idea (11, 17).

Dexamethasone has been shown to have a profound negative effect (three- to fourfold decrease in activity) on LR promoter activity in bovine cells (9). Thus, the transient decrease in LR RNA-containing neurons following dexamethasone treatment is best explained as downregulation of LR RNA transcription in neurons to an undetectable level. The fact that approximately 40% of LR RNA-containing cells appear to be unaffected after treatment might be due to a requirement for other preexisting cellular conditions to realize the dexamethasone effect or, alternatively, due to large variations in steady-state levels of LR RNA in individual cells. Because LR RNA almost completely overlaps an

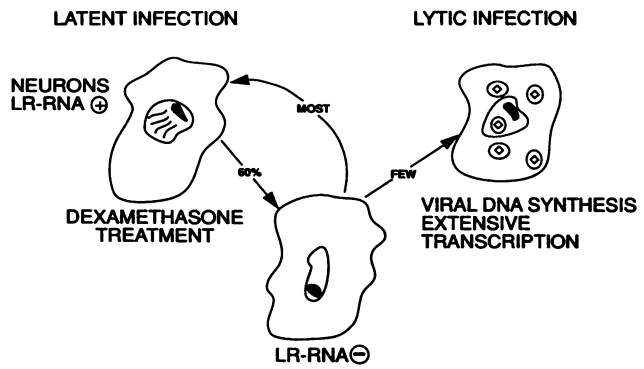

FIG. 3. Proposed model: downregulation of LR RNA expression is necessary but not sufficient for viral reactivation. LR RNA is downregulated in a significant proportion of latently infected cells following dexamethasone treatment (60%); however, only a small number of these LR RNA-negative cells—those in which some other necessary reactivation requirement (cellular or viral) has been met—actually go on to reactivate latent virus (see Discussion).

IE gene present on the complementary strand—a gene that might be expected to be active during viral reactivation—it is possible, although less likely, given the kinetics of viral transcriptional changes, that the decreases of LR RNA at 24 and 48 h PT are due to competition between newly synthesized IE gene RNA and the labelled DNA probe during in situ hybridization.

The significance of this LR RNA decrease to viral reactivation is unknown. However, the decrease is concomitant with the earliest ganglionic changes and is positively correlated with viral reactivation. These observations suggest that altered regulation of LR RNA transcription is a significant event in latent viral reactivation, either causally related to reactivation or a direct consequence of it.

Interestingly, the decrease in LR RNA-containing neurons is transient; numbers return to near-normal levels by 72 h PT. It seems unlikely, given temporal considerations, that this is due to infection of additional neurons which would result from ocular replication of reactivated virus with subsequent spread of virus back to the ganglion. If it were, reactivated virus appearing at the ocular site, at the very earliest between 24 and 48 h PT, would have to replicate at the ocular site, be transported back to ganglionic neurons, and express detectable levels of LR RNA by 72 h PT. A more plausible explanation is that the dexamethasone effect on LR RNA is transient and that LR RNA is reexpressed in a majority of the cells that had become LR RNA negative following treatment (as shown in Fig. 3). If this interpretation is correct, and if virus reactivates from neurons that are LR RNA positive prior to treatment, it is tempting to speculate about a role for LR RNA in viral reactivation. These data support a model in which downregulation of LR RNA expression is necessary but not sufficient for viral reactivation. Here, LR RNA is downregulated in a significant proportion of latently infected cells after dexamethasone treatment (60%); however, only a small number of these LR RNA-negative cells, those in which some other necessary reactivation requirement has been met (cellular or viral), actually go on to reactivate latent virus, while a majority of them cycle back and reexpress LR RNA. This explanation is consistent with the quantitative results that indicate that, at most, only a fraction of all LR RNA-containing neurons show evidence of viral reactivation (extensive transcriptional changes or the presence of viral DNA) following treatment.

The relatively synchronous reactivation obtained in this study with the BHV-1 rabbit latency model and dexamethasone suggests the usefulness of the model for further studying the molecular mechanisms underlying viral reactivation in vivo.

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REFERENCES

1. **Baumann, H., G. L. Firestone, T. L. Burgess, K. W. Gross, K. R. Yamamoto, and W. A. Held.** 1983. Dexamethasone regulation of α_1-acid glycoprotein and other acute phase reactants in rat liver and hepatoma cells. J. Biol. Chem. **258**:563–570.
2. **Bitsch, V.** 1973. Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection. Appl. Microbiol. **26**:337–343.
3. **Brahic, M., and A. T. Haase.** 1978. Detection of viral sequences of low reiteration frequency by *in situ* hybridization. Proc. Natl. Acad. Sci. USA **75**:6125–6129.
4. **Brown, G. A., and H. J. Field.** 1990. Experimental reactivation of bovine herpesvirus 1 (BHV-1) by means of corticosteroids in an intranasal rabbit model. Arch. Virol. **112**:81–101.
5. **Davies, D. H., and L. E. Carmichael.** 1973. Role of cell-mediated immunity in the recovery of cattle from primary and recurrent infections with infectious bovine rhinotracheitis virus. Infect. Immun. **8**:510–518.
6. **Davies, D. H., and J. R. Duncan.** 1974. The pathogenesis of recurrent infections with infectious bovine rhinotracheitis virus induced by calves by treatment with corticosteroids. Cornell Vet. **64**:340–366.
7. **Homan, E. J., and B. C. Easterday.** 1980. Isolation of bovine herpesvirus-1 from trigeminal ganglia of clinically normal cattle. Am. J. Vet. Res. **41**:1212–1213.
8. **Huck, R. A., P. G. Miller, and D. G. Woods.** 1973. Experimental infection of maiden heifers by the vagina with infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus. J. Comp. Pathol. **83**:271–279.
9. **Jones, C., G. Delhon, A. Bratanich, G. Kutish, and D. Rock.** 1990. Analysis of the transcriptional promoter which regulates the latency-related transcript of bovine herpesvirus 1. J. Virol. **64**:1164–1170.
10. **Kahrs, R. F.** 1981. Viral diseases of cattle, p. 135–156. Iowa State University Press, Ames.
11. **Kubin, G.** 1969. Intermittent recovery of IPV virus from a naturally-infected bull. Wien. Tieraerztl. Monatsschr. **56**:336–337.
12. **Kutish, G., T. Mainprize, and D. Rock.** 1990. Characterization of the latency-related transcriptionally active region of the bovine herpesvirus 1 genome. J. Virol. **64**:5730–5737.
13. **Narita, M., S. Inui, K. Namba, and Y. Shimizu.** 1976. Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with infectious bovine rhinotracheitis virus. J. Comp. Pathol. **86**:93–100.
14. **Narita, M., S. Inui, K. Namba, and Y. Shimizu.** 1978. Neural changes in calves after intraconjunctival inoculation with infectious bovine rhinotracheitis virus. J. Comp. Pathol. **88**:387–394.
15. **Narita, M., S. Inui, K. Namba, and Y. Shimizu.** 1978. Neural changes in recurrent infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. Am. J. Vet. Res. **39**:1399–1403.
16. **Narita, M., S. Inui, K. Namba, and Y. Shimizu.** 1981. Recrudescence of infectious bovine rhinotracheitis virus and associated neural changes in calves treated with dexamethasone. Am. J. Vet. Res. **42**:1192–1197.
17. **Rock, D. L., S. L. Beam, and J. E. Mayfield.** 1987. Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. J. Virol. **61**:3827–3831.

18. **Rock, D. L., W. A. Hagemoser, F. A. Osorio, and D. E. Reed.** 1986. Detection of bovine herpesvirus type 1 RNA in trigeminal ganglia of latently infected rabbits by *in situ* hybridization. J. Gen. Virol. **67**:2515–2520.
19. **Rock, D. L., and D. E. Reed.** 1982. Persistent infection with bovine herpesvirus type 1: rabbit model. Infect. Immun. **35**:371–373.
20. **Rousseau, G. G., and J. D. Baxter.** 1979. Glucocorticoid hormone action. Springer-Verlag, Heidelberg, Germany.
21. **Sheffy, B. E., and D. H. Davies.** 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. Proc. Soc. Exp. Biol. Med. **140**:974–976.
22. **Snowdon, W. A.** 1965. The IBR-IPV: reaction to infection and intermittent recovery of virus from experimentally infected cattle. Aust. Vet. J. **41**:135–142.
23. **Stroop, W. G., D. L. Rock, and N. W. Fraser.** 1984. Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by *in situ* hybridization. Lab. Invest. **51**:27–38.
24. **Wirth, U. V., K. Gunkel, M. Engels, and M. Schwyzer.** 1989. Spatial and temporal distribution of bovine herpesvirus 1 transcripts. J. Virol. **63**:4882–4889.
25. **Yamamoto, K. R.** 1985. Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. **19**:209–215.