Reactivation of a Bovine Herpesvirus After Corticosteroid Treatment[1] (36592)

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Herpes simplex is the classical example of a persistent, recurrent viral disease. Reactivation of herpes simplex virus (HSV) has been demonstrated in man and laboratory hosts by various artificial stimuli (1–4).

Infectious bovine rhinotracheitis virus (IBR), an herpesvirus infection of cattle has been suggested to be another example of a persistent herpesvirus infection, with recrudescence occurring after "stress" in a manner analogous to herpes simplex virus infection in man. IBR virus has been recovered from experimentally infected cattle, intermittently, for as long as 578 days after infection (5). These animals had neutralizing antibody titers at the time of recrudescence of the virus. Sporadic reexcretion of virus following what was presumably natural infection, and possible reactivation by corticosteroids has also been reported (6, 7).

This report concerns reactivation of IBR virus in cattle that had serum neutralizing antibody titers by the administration of synthetic corticosteroids, and the tissues from which virus was recovered following drug treatment.

Materials and Methods. Animals used in this study were 9 adult Holstein bulls which had been shown to have neutralizing antibody titers to IBR virus for at least 4 years, indicating past exposure to the virus, time and route of infection were unknown. In addition, 19 Holstein heifers were examined at 3 to 9 months after intranasal infection with 10^7 $TCID_{50}$ of virulent IBR virus (Burroughs strain). Eleven of these heifers had been previously immunized with a modified live IBR virus vaccine containing approximately 10^4 $TCID_{50}$, 32nd passage (Colorado strain) given by the intranasal or intramuscular route; 8 had not been vaccinated and were serologically negative at the time of exposure to virulent virus. The heifers were approximately 18 months of age, and were pregnant at the time of infection with virulent virus. All animals had serum neutralizing antibody at the time of examination, titers ranged from $-\log 10^{1.2}$ to $-\log 10^{2.2}$ per 0.1 ml of serum.

Corticosteroid treatment. Thirteen heifers were inoculated intravenously with 20 mg dexamethasone (Azium, Schering) daily for 6 or 7 days; 6 heifers were slaughtered without previous corticosteroid treatment. Six bulls were given 40 mg dexamethasone daily for 7 days, and three were slaughtered without treatment. All treated animals and untreated control animals were slaughtered 48 hr after the last corticosteroid treatment.

Virus isolation. Representative portions of the following tissues were collected at slaughter: Upper respiratory tract (nasal mucosa and larynx), lower respiratory tract (trachea and lung), adrenal, central nervous system (cerebrum, medulla and trigeminal ganglion), and reproductive tract (ovary, uterine and vaginal mucosae or testis, epididymis, penis, and prepuce). Two pieces (approx 3 mm^3) of each tissue were placed in 25 cm^2 plastic flasks, (Falcon) and maintained in McCoys 5A medium (Microbiological Associates) with 40% bovine serum plus antibiotics at three times usual concentration at 37°. Organ culture medium was changed after 4, 24 and 96 hr and then at weekly intervals for 2 to 4 weeks. Fluid collected at 96 hr and later was assayed for the presence of IBR virus by inoculation of 0.5 ml of fluid into each of two tubes of monolayer bovine testic-

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ular (BT) cells. Tubes were frozen and thawed after 6 days incubation at 37° and passaged once before they were considered negative.

Nasal and conjunctival swab specimens were collected before treatment and thereafter at intervals from infected and control cattle. Swabs were placed in 2.0 ml of Earles balanced salt solution containing 0.5% lactalbumin hydrolysate plus antibiotics. Bovine testicular cell cultures in tubes were overlayed with 0.5 ml of this fluid and incubated for 30 min at room temperature. They then were washed twice with phosphate buffered saline and maintenance medium was replaced.

Monolayer cultures of primary BT cells were prepared by standard tissue culture techniques.

Results. Administration of corticosteroids resulted in the reexcretion of IBR virus in nasal secretions of 18/19 animals, and in conjunctival secretions of 8/13, beginning 3 to 5 days after commencement of steroid treatment. No IBR virus was recovered from the secretions of untreated animals, nor from secretions collected before treatment (Table I). Virus was recovered from organ cultures of tissues of the upper respiratory tract of 12/19 animals, and irregularly from the lower respiratory tract, the reproductive tract, adrenal glands and central nervous system (Table II). Vaccinal status, pregnancy, sex or time between infection and study did not affect the pattern of virus recovery. IBR virus was recovered from the larynx and ovary of one nontreated nonvaccinated heifer slaughtered 3 months postinfection. IBR virus was not recovered from the tissues or secretions of any other untreated animal.

Discussion. IBR virus and herpes simplex virus have many similarities both in pathogenesis and disease syndromes produced. Both viruses have a predilection for tissues derived from embryonic ectoderm, producing lesions on the mucous membranes of the mouth, eyes and genital tracts. The respiratory and genital syndromes in man are caused by distinct serotypes, HSV-1 and HSV-2 respectively (8). Although independent respiratory and genital syndromes are known in cattle, no significant differences between virus strains have been demonstrated (9, 10). In addition to local infections of mucous membrances in older animals, both viruses cause acute, generalized, usually fatal infections in newborns (2, 11) and encephalitis (2, 12–14).

Latent infections with spontaneous reexcretion of the virus have been demonstrated following natural and experimental infection with HSV (15, 16) and IBR virus (5). Reactivation of HSV in man has been associated with natural and artificially induced fever,

TABLE I. Recovery of IBR Virus from Nasal and Conjunctival Secretions of Persistently Infected Cattle Following Treatment with Corticosteroids.

Treatment:	Heifers				Bulls	
	Vaccine + virulent virus[a]		Unvaccinated + virulent virus[b]		IBR[c] serologically positive	
Specimens	Treated	Untreated	Treated	Untreated	Treated	Untreated
Before treatment						
Nasal secretions	0/8[d]	0/3	0/5	0/3	0/6	0/3
Conjunctival secretions	0/8	0/3	0/5	0/3	NT[e]	NT
After treatment						
Nasal secretions	8/8	0/3	5/5	0/3	5/6	0/3
Conjunctival secretions	5/8	0/3	3/5	0/3	NT	NT

[a] Immunized with commercial IBR vaccine, then exposed to virulent IBR virus, Burroughs strain.
[b] Virulent IBR virus Burroughs strain only.
[c] Route and time of exposure unknown, but presumably natural infection at least 4 years previously.
[d] Number positive/number examined.
[e] NT = not tested.

TABLE II. Recovery of IBR Virus from Tissues of Cattle After Treatment with Corticosteroids.

Treatment: / Specimens	Heifers				Bulls	
	Vaccine + virulent virus[a]		Unvaccinated + virulent virus[b]		IBR[c] serologically positive	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Upper respiratory	6/8[d]	0/3	4/5	1/3	2/6	0/3
Lower respiratory	3/8	0/3	2/5	0/2	0/6	0/3
Central nervous system	1/7	0/3	2/4	0/2	0/2	NT
Adrenal	1/8	0/3	1/5	0/2	2/6	0/3
Reproductive tract	0/8	0/3	1/5	1/3	1/6	0/3

[a-d] See footnotes, Table I.

exposure to UV light, strong wind, menstruation, administration of foreign proteins, emotional stress, posterior root section of the fifth cranial nerve, and local application of corticosteroid hormones to the cornea (3). This report demonstrates that in addition to many other similarities to HSV, IBR virus can cause persistent or latent infections in the presence of neutralizing antibody. These infections can be reactivated by synthetic corticosteroid hormones, causing not only reexcretion of the virus in secretions of the upper respiratory tract but also reactivation of infection in a variety of other organ systems.

Herpes simplex virus has been suggested to persist as a chronic low grade infection of tissues associated with the mouth, eyes, and upper respiratory tract, or as a truly latent infection of the trigeminal ganglion (1–3). One cannot determine from this study whether either of these possible mechanisms applies in cattle, however, further use and refinement of these techniques may allow a more precise determination of the site and possibly the form of persistent herpesvirus infections in a natural host.

Summary. Consistent recrudescence of IBR virus from latently infected cattle following a series of inoculations of a synthetic corticosteroid has been demonstrated. In addition IBR virus was frequently recovered from the tissues of the upper respiratory tract, and irregularly in a number of other organs by organ culture techniques. While IBR virus was recovered from all treated animals it was never recovered from nasal and conjunctival secretions of control animals or from treated animals until after 3 days of steroid inoculations and was recovered from tissues of only 1/9 untreated animals.

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