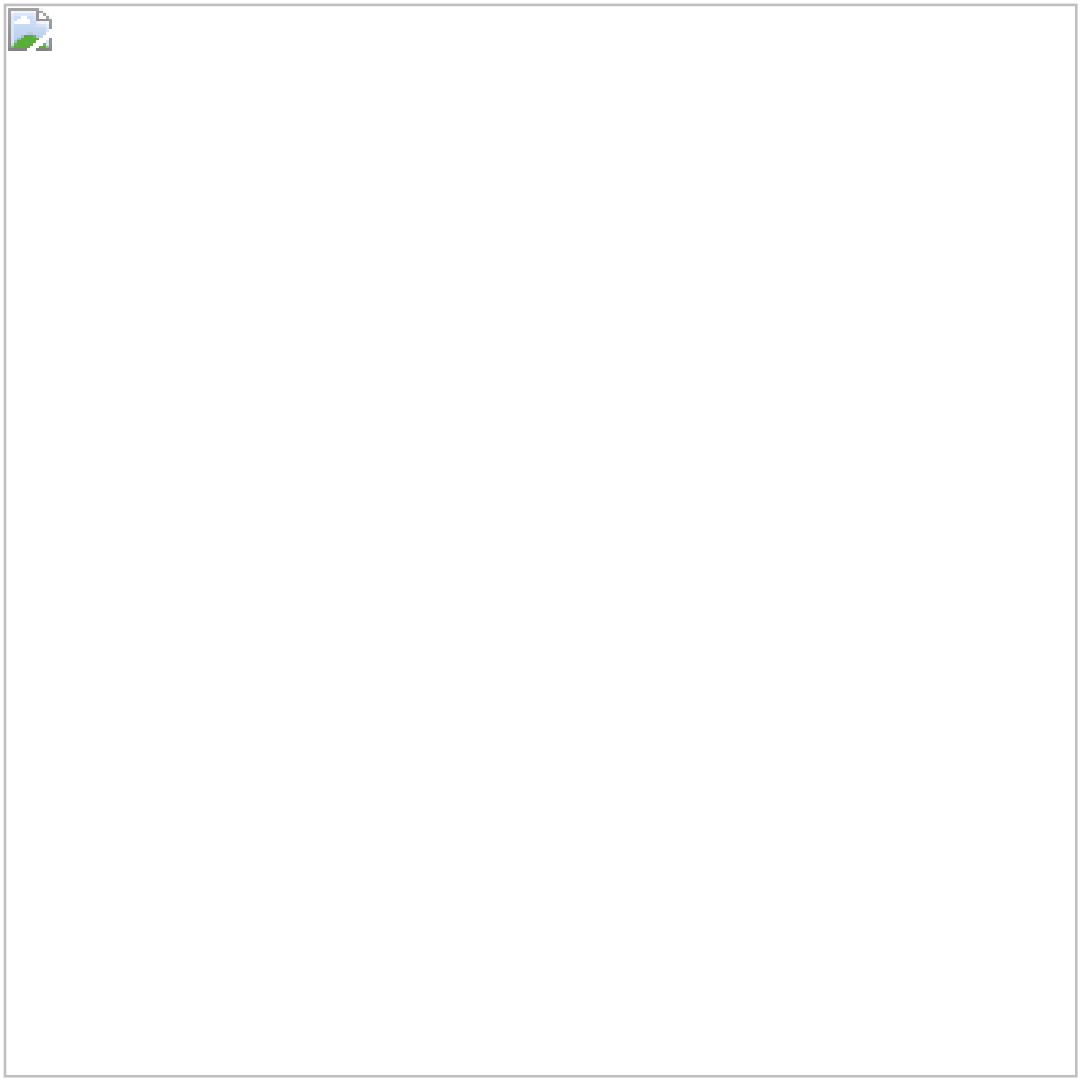

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ISOLATION OF A RETROVIRUS FROM THE AMERICAN BISON AND ITS RELATION TO BOVINE RETROVIRUSES

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ABSTRACT: Tissue samples were removed at necropsy from five American bison (*Bison bison*) with clinical signs of a disease resembling malignant catarrhal fever (MCF). Using cell-associated virus techniques, attempts were made to isolate viruses from these tissues by culturing them directly or by co-culture with bovine fetal cells. Among the viruses isolated was one which was syncytiogenic and multiplied in bovine fetal spleen cells and remained highly cell-associated. The presence of reverse transcriptase activity indicated that it was a retrovirus. Also, it had antigenic cross activity with bovine syncytial virus, but not with bovine leukemia or bovine maedi-like retroviruses. We do not attribute a direct causative role of this retrovirus to MCF, but indirect relationships are possible.

INTRODUCTION

Although several viral infections have been documented for Indian (*Bubalus bubalis*) and African (*Syncerus caffer*) buffaloes (Hamblin and Hedgar, 1982; Persechino et al., 1983; Samara and Pinto, 1983; Tongaonkar et al., 1983), few have been reported for the American bison. Some diseases of the American bison are considered to be similar to those of cattle. For example, malignant catarrhal fever (MCF) has been described recently as a disease of importance of the American bison and is highly fatal (Liggitt et al., 1980; Todd and Storz, 1983). In cattle, MCF is one of the cell-associated viral infections which is transmitted by lymphocytes. This may also be true in the American bison (Clarke et al., 1973; Liggitt et al., 1980; Samara and Pinto, 1983). In an attempt to isolate the causative virus, tissue samples from five MCF-affected American bison of three different herds in Colorado and Wyoming were examined in 1975 and 1976 by applying techniques used principally for cell-associated viruses. Using this approach, which also detects cytocidal viruses, several viral isolates were obtained. These included: cytomegaloviruses (Storz et al., 1984) and syncytiogenic retroviruses. Some details on isolation and on properties of the bison syncytiogenic retrovirus 76-P-5523 and its antigenic relationship to bovine retroviruses are described in this report.

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MATERIALS AND METHODS

Collection and processing of specimens: Samples of spleen, kidneys, testicles, thyroid, and adrenal glands were taken aseptically at necropsy from five American bison affected with a disease resembling bovine MCF in clinical signs. The samples were transported on ice to the laboratory. Blood was also obtained for serum preparation and for harvest of buffy coat cells.

Culture for cell-associated viruses: Direct culture of intact cells from tissue specimens of the affected bison and co-culture of such cells with low passage bovine fetal spleen (BFS) and bovine fetal thyroid cells (BFThy) were used to detect cell-associated viruses.

Cells were dispersed from the tissue samples by trypsin digestion and were propagated using Eagle's minimum essential medium (MEM) containing 10% heat-inactivated bovine fetal serum (BFS) and antibiotics (100 IU/ml of penicillin G and 100 mcg/ml streptomycin sulfate).

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Unattached cells were removed during the first medium change after 3 days. When cells had grown to monolayers, they were dispersed by trypsin-EDTA and divided for subpassage. The cells were maintained and examined microscopically for 2 to 3 mo.

In the co-culture procedure, suspensions of trypsin-dispersed cells of MCF-affected bison were either seeded onto cell monolayers of BFS or BFThys cells, or they were added to suspensions of these cells immediately before seeding. These cells were cultured with lactalbumin-vitamin (Lavit) medium containing streptomycin (500 mcg/ml), penicillin (500 IU/ml), and 10% inactivated BFS. Microscopic examinations were made each day to detect cytopathic changes. At intervals of 2 to 3 wk, or after extensive fusion of cells had occurred, subpassages were made. When polykaryons were observed, a 10th of the fused cell culture was inoculated onto fresh monolayers. Representatives of the polykaryon-forming isolates were frozen at −70 C in Lavit medium containing 10% BFS and 10% glycerol or 7% dimethylsulfoxide. When infected cells were subsequently thawed, they were cultured in MEM containing 10% BFS, 25 mM HEPES buffer, and antibiotics.

Indirect fluorescent test: To determine the antigenic relationship of these viral isolates to other known viruses an indirect fluorescent antibody (IFA) test was employed (Malmquist et al., 1969). The syncytiogenic viral isolates were propagated in BFS cells on coverslips in 35 mm culture plates or eight-chamber slides (Lab-Tek, Napierville, Illinois) until syncytia developed. All cell preparations were then fixed in paraformaldehyde. BFS cells infected with either bovine syncytial virus (BSV), bovine respiratory syncytial virus (BRSV), bovine morbilli-like virus (72P-635), bovine parainfluenza virus (PI_3), bovine maedi-like virus (BMV), or bovine leukemia virus (BLV) were included as controls. Antisera against BLV, BMV, and BSV were kindly provided by Dr. M. J. Van Der Maaten of the National Animal Disease Center (Ames, Iowa 50010, USA). Antisera against other bovine viruses were also used. Rabbit anti-bovine IgG (heavy and light chain specific) conjugated with fluorescein isothiocyanate (Cappel Laboratories, Malvern, Pennsylvania 19380, USA) was used for the IFA tests.

Cytology and rate of polykaryon formation: BFS and other cell cultures in 35 mm culture plates were infected with the syncytiogenic isolate 76-P-5523. At intervals of 24 hr cultures were fixed by the Bouin's technique, and stained with the Giemsa method. The number of nuclei per polykaryon was counted and the cytological features of cytoplasm and nuclei were assessed. The range of different cell types recruited into polykaryons by the viral isolates also was determined.

Reverse transcriptase: To determine if 76-P-5523 was a retrovirus, reverse transcriptase (RT) assays were done using standard procedures described for bovine leukemia virus (Graves et al., 1977) with modifications suggested by Collaborative Research, Inc. (Waltham, Massachusetts 02154, USA). Culture fluid or cellular lysates from infected and control cell cultures were analyzed for RT activity in the presence of Mg^{++} or Mn^{++}. The cell culture fluid was centrifuged to remove cellular debris (9,000 *g*, 20 min) and then centrifuged at 100,000 *g* for 60 min to pellet the virus. The trypsinized cell monolayers were then frozen and thawed three times to rupture the cells and centrifuged as described above. Culture fluid from fetal lamb kidney cells infected with BLV was included as a positive control.

RESULTS

Recovery of cell-associated viruses: Co-cultures of BFS and BFThy with cells dispersed from the spleen and the thyroid gland of Bison 76-P-5523 resulted in the isolation of syncytiogenic viruses. Control cultures did not develop syncytia. Cell fusion was observed 13 and 19 days in the first passage, respectively, after co-culture of bison spleen cells with BFS or BFThys. Similar changes developed in co-cultures of thyroid cells from this bison with BFThys cells during the fourth passage involving 42 days of culture. Adrenal cells of this bison did not induce changes in co-culture. The direct cultures of cells from these organs of the bison did not grow well and could not be maintained longer than 9 to 20 days.

The cells dispersed from three other bison did not induce changes in the co-culture systems described above, but those of bison 75-P-2756 yielded an infectious agent with cytomegalovirus properties as reported elsewhere (Todd and Storz, 1983).

Cell-associated nature of isolate 76-P-5523: Infectivity and fusion activity of strain 76-P-5523 remained associated with

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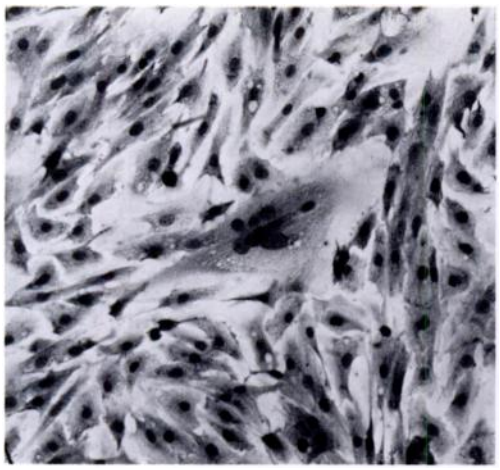

FIGURE 1. Polykaryons formed by co-culture of American bison isolate 76-P-5523 cells with bovine fetal spleen cells. Passage 5. ×83.

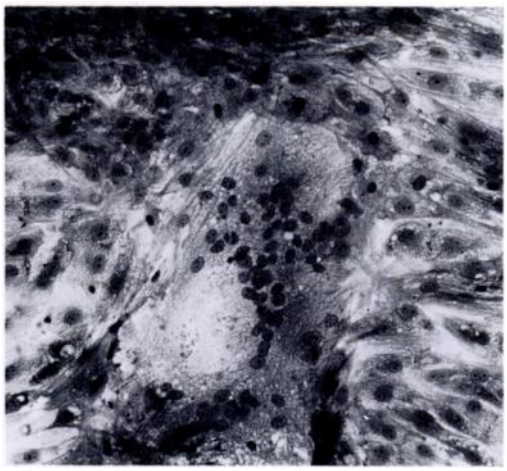

FIGURE 2. Polykaryons formed by co-culture of American bison isolate 76-P-5523 with bovine fetal spleen cells. Passage 25. ×87.

the cellular fractions. The cell culture fluid from infected cells was not infective following low speed centrifugation (2,000 *g* for 15 min) to remove cellular components. Freezing and thawing of the cellular fraction reduced infectivity. Infectivity was maintained if infected BFS cells were frozen with the addition of glycerol (10%) or dimethyl sulfoxide (7%) to the medium to retain cell viability during long term storage in liquid nitrogen.

Cytological features of infected cells: The size of the polykaryons was usually uniform in a given culture and the cells contained smaller numbers of nuclei (up to 15) during the initial passages (Fig. 1). This is also a characteristic of other bovine retroviruses such as BLV and BMV (Van Der Maaten et al., 1972; Graves and Jones, 1981). After adaptation to BFS cells, the number of nuclei per polykaryon increased (Fig. 2). The rate of cell recruitment based on the daily examination of 100 stained polykaryons is given in Table 1. Intranuclear or cytoplasmic inclusions were not detected by light microscopic examination.

Reverse transcriptase as a characteristic of isolate 76-P-5523: From the results presented in Table 2, it appears that strain 76-P-5523 is a highly cell-associated virus with a RT cation requirement for Mg^{++}. This enzyme function was detected in both the extracellular viral preparations and in the cytoplasmic lysate. The supernatant fluid from the BLV-infected control cell line had the expected RT with preferential Mg^{++} requirement (Graves et al., 1977).

Antigenic relationship of isolate 76-P-5523 to other bovine viruses: BFS cells infected with strain 76-P-5523 had transient nuclear fluorescence and strong cytoplasmic fluorescence with antiserum

TABLE 1. Number of nuclei per polykaryon produced by co-cultivation of American bison isolate 76-P-5523-infected cells with bovine fetal spleen cells.

Days of incubation	Average number of nuclei (range)*
1	8 (5–24)
2	11 (6–30)
3	17 (8–50)
4	14 (6–36)
5	13 (7–47)
6	15 (7–55)
7	13 (7–41)

* Based on the examination of 100 polykaryons.

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TABLE 2. Viral reverse transcriptase activity in American bison isolate 76-P-5523-infected bovine fetal spleen cells and supernatant fluids.

Sample	Cation supplement	
	Mg^{++}	Mn^{++}
BFS cells infected with strain 76-P-5523		
Cell-free culture fluid	15.3[a]	<1.0
Cellular lysate	83.2	1.3
Non-infected BFS control cells		
Cell-free culture fluid	<1.0	<1.0
Cellular lysate	<1.0	<1.0
Fetal lamb kidney cells persistently infected with BLV		
Cell-free culture fluid	48.8	12.6

[a] Picomoles of ^{3}H-thymidine 5'-triphosphate incorporated in samples obtained from 25-cm^2 culture flask.

against BSV. No specific fluorescence was obtained with strain 76-P-5523-infected cells and antibodies against two additional bovine retroviruses, BLV and BMV, nor antibodies to other bovine syncytiogenic agents. Normal bovine serum also gave negative IFA tests with infected and uninfected cells (Table 3). The IFA tests with bison 76-P-5523 serum were positive at 1:8 when tested with cells infected with the bison agent in initial primary co-cultures. Following long term storage of the serum 15 at −20 C, the IFA reactivity was lost. This difference may have derived from the use of a different rabbit anti-bovine IgG rather than an anti-bison IgG in the IFA tests. This bison serum did not react with BLV- or BMV-infected cells.

DISCUSSION

The syncytiogenic viral agent recovered from spleen and thyroid gland cells of an American bison has the viral reverse transcriptase activity which is a characteristic of retroviruses. It is related to the BSV as shown by the antigenic cross reactivity, and the highly cell-associated nature of the agent (Clarke and MacFerran, 1970).

This virus originated from the bison affected with MCF because the stocks of BFS and BFThy cultures used remained normal over extended periods of observation. Bovine syncytial viruses have been recovered previously from MCF-affected cattle (Clarke et al., 1973; Liggitt et al., 1980). Bovine syncytial viruses also have been detected in normal and compromised cattle affected with lymphosarcoma (Malmquist et al., 1969). These viruses are closely cell-associated and cannot be detected if cell-free virus isolation techniques are applied.

The clear antigenic relationship of bison and bovine syncytial viruses is also of

TABLE 3. Antigenic relationship of American bison isolate 76-P-5523 with other polykaryon-inducing bovine viruses as determined by the indirect fluorescent antibody test.

Bovine fetal spleen cells infected with	Antiserum against[a]						
	BSV	BRSV	72-P-535	PI_3	BMV	BLV	Control
76-P-5523	+[b]	−	−	−	−	−	−
BSV	+	−	−	−	−	−	−
BRSV	−	+	−	−	−	−	−
72-P-535	−	−	+	−	−	−	−
PI_3	−	−	−	+	−	−	−
BMV	−	−	−	−	+	−	−
BLV	−	−	−	−	−	+	−
BFS control	−	−	−	−	−	−	−

[a] Sera containing antibody to: BSV = bovine syncytial virus; BRSV = bovine respiratory syncytial virus; 72-P-535 = bovine morbilli-like virus; PI_3 = bovine parainfluenza virus, type 3; BMV = bovine maedi-like virus; BLV = bovine leukemia virus.
[b] + = positive in fluorescent antibody test; − = negative in fluorescent antibody test.

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interest. The significant question remains whether bison became infected from cattle or whether this infection evolved independently in these two bovid species. The prevalence of this infection in bison should be explored. The mode of transmission of this closely cell-associated virus is not defined clearly but, in the case of bovine retroviruses, the association appears to be with the lymphocytes (Graves and Jones, 1981). Experimental transmission of the infection by lymphocyte inoculation has been reported (Van Der Maaten et al., 1972). Additional types of retroviruses have not been isolated from bison. Interestingly, other viruses such as the cytomegaloviruses recovered from cattle and bison are also indistinguishable on the basis of antigenicity and restriction enzyme analysis of their DNA genome (Storz et al., 1984).

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