

# Effect of bovine immunodeficiency-like virus infection on immune function in experimentally infected cattle

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## ABSTRACT

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Bovine immunodeficiency-like virus (BIV) is a bovine lentivirus that has antigenic and genetic homology with the human immunodeficiency virus. Little work has been reported on the effect of BIV infection on bovine immune function. This study was designed to evaluate lymphocyte blastogenesis, mononuclear cell subset numbers, neutrophil function, hematology, and clinical signs in three groups of cattle. These groups were evaluated at 0–2 months post inoculation (PI, Group 1), 4–5 months PI (Group 2), or 19–27 months PI (Group 3). BIV infected animals were inoculated with the R-29 isolate of BIV in tissue culture cells, peripheral blood mononuclear cells from a R-29 infected calf, or a molecular clone of the R-29 isolate. Most inoculated animals seroconverted to BIV by Western immunoblot. BIV was reisolated from most of the animals inoculated. BIV infection was associated with an increase in the lymphocyte blastogenic response to the mitogen phytohemagglutinin in Groups 2 and 3. Neutrophil antibody dependent cell mediated cytotoxicity and neutrophil iodination were decreased ( $P < 0.05$ ) in BIV infected cattle (Groups 2 and 3 and Group 3, respectively). All animals were clinically normal during the evaluation periods. Notable differences were not observed in the other assessments performed. Work with additional BIV isolates and over longer time frames is warranted.

## ABBREVIATIONS

ADCC, antibody-dependent cell-mediated cytotoxicity; AICC, antibody-independent cell-mediated cytotoxicity; BIV, bovine immunodeficiency-like virus; BLV, bovine leukemia virus; Bo, bovine; BSV, bovine syncytial virus; BVDV, bovine viral diarrhea virus; FBL, fetal bovine lung; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin.

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## INTRODUCTION

Bovine immunodeficiency-like virus (BIV) is a bovine retrovirus of the lentivirus subfamily that was first isolated from a dairy cow, R-29, with persistent lymphocytosis, progressive weakness and emaciation. At necropsy lymph node hyperplasia and lymphocytic perivascular cuffing in the brain were noted. Inoculation of calves with the R-29 isolate resulted in enlargement and follicular hyperplasia of lymph nodes as well as a transient lymphocytosis (Van der Maaten et al., 1972). In serologic surveys, conducted on approximately 2200 cattle in the southern USA, approximately 4% of the cattle tested had antibody against BIV (Amborski et al., 1989; Black, 1990). BIV has structural and antigenic similarity to the human immunodeficiency virus (HIV). Western immunoblot analysis and indirect immunofluorescence testing indicate shared antigenic epitopes of the *gag* (core) proteins of HIV and BIV. Sequence analysis of the 5' end of the BIV *pol* gene (which codes for reverse transcriptase) indicates 60% nucleotide sequence homology with other lentiviruses (HIV-1, equine infectious anemia virus, Visna virus, and caprine arthritis-encephalitis virus) (Gonda et al., 1987).

The culmination of HIV infection is clinically apparent as the acquired immune deficiency syndrome (AIDS). Yet, HIV infection does produce detectable changes in the host prior to the onset of AIDS. Infection with HIV has been associated with an acute 'flu-like' syndrome shortly after seroconversion (Cooper et al., 1985). A decrease in CD4<sup>+</sup> lymphocytes and an increase in CD8<sup>+</sup> lymphocytes in the blood have been detected by 6 months after seroconversion to HIV (Giorgi et al., 1987; Giorgi and Detels, 1989). B cell antibody production was reported to be compromised beginning at seroconversion, while T cell proliferative responses remained normal during the first 10 months (Terpstra et al., 1989). Other workers reported decreased lymphocyte blastogenic responses to mitogens in HIV infected individuals who were clinically staged at WR1 (seropositive, clinically normal) with no estimate of the period of infection (Ridley et al., 1989). There have been few reports on the immunological effects of BIV infection in cattle. Interestingly, two calves were reported to have a selective depletion of CD4<sup>+</sup> cells 3–4 months after inoculation with the original R-29 strain of BIV (Stott et al., 1989). Infection with BIV was also associated with decreased blastogenesis to mitogens in two calves 6 months after infection (Martin et al., 1991). Thus, there is evidence that measurable immune function changes following both HIV and BIV infection are present within a relatively short period of time after infection. The present study was designed to evaluate the effect of BIV inoculation on several measures of immune function in three groups of cattle at 0–2, 4–5, and 19–27 months post inoculation (PI). These three groups were used to define an optimal challenge inoculum for BIV and to characterize early pathogenic effects of BIV infection.

## MATERIALS AND METHODS

### *Animals*

#### *Group 1: animals 0–2 months PI*

Eight male Holstein calves, aged 2–4 months, were randomly assigned to either control ( $n=3$ ) or BIV ( $n=5$ ) inoculated groups. The control and inoculated calves were housed in separate rooms inside isolation facilities at the Veterinary Medical Research Institute, Ames, IA. All of the animals were tested on Days  $-24$  and  $60$  for antibodies to bovine syncytial virus (BSV), and bovine leukemia virus (BLV) by agar gel immunodiffusion (Malmquist et al., 1969; Miller and Van der Maaten 1976), and to BIV by Western blot analysis (Whetstone et al., 1991). Two animals appeared to have maternal antibodies to BLV or BSV. These animals were weakly positive when initially tested, but 85 days later the same animals were negative for both viruses. All of the calves were seronegative for BIV before inoculation and were seronegative for BLV and BSV at 60 days PI. Of the five BIV inoculated animals, only three developed antibody against BIV. The results presented are from only the three BIV antibody positive animals.

#### *Inoculum for Group 1 animals*

Cell culture material infected with BIV, isolate R-29, was inoculated into a Holstein calf aged  $<3$  months. The BIV was then serially passed through three more calves by directly transfusing blood from an infected calf to a non-infected calf as previously described (Carpenter et al., 1992). This serial passage was done in an attempt to increase the virulence of the R-29 isolate and to obtain a challenge inoculum that was free of bovine viral diarrhea virus (BVDV), which is known to contaminate the R-29 isolate. Peripheral blood mononuclear cells (PBMC) from the last infected calf were isolated by density gradient centrifugation, resuspended in a solution containing 81% Eagle's minimum essential-medium, 9% fetal bovine serum, and 10% dimethyl sulfoxide at a concentration of  $2.5 \times 10^5$  cells  $\text{ml}^{-1}$ . The cells were frozen in a stepwise fashion: 12 h at  $-20^\circ\text{C}$ , 24 h at  $-70^\circ\text{C}$ , and then stored in liquid nitrogen. The inoculum produced BIV syncytia in cell culture, but did not contain BVDV when tested by indirect immunofluorescence assay. The frozen inoculum was rapidly thawed immediately prior to intravenous inoculation into the calves. Each of the five calves in the BIV group received  $2.5 \times 10^7$  PBMC intravenously on Day 0 of the experiment. Control animals received a like number of similarly treated cells from a BIV negative animal.

#### *Sample collection for Group 1 animals*

Blood was collected two to three times a week for hematologic evaluation during Weeks  $-3$  through 10. Blood (250 ml) was collected twice per week

in Weeks -1 to 8, for in vitro immune function analysis (described below). Mononuclear cell subset analysis and virus isolation were performed once a week. The animals were observed daily, and rectal temperatures were recorded three times a week.

#### *Group 2: animals 4–5 months PI*

Nine male Holstein calves, 2–4 months old, were randomly assigned to either control (five animals) or BIV inoculated (four animals) groups. All animals were free of antibody to BIV (Western blot analysis), and BLV (agar gel immunodiffusion) at the beginning of the experiment. All of the animals but one BIV infected calf were free of antibody to BSV. The animals were housed together by group with the groups physically separated.

#### *Inoculum for Group 2 animals*

The four BIV inoculated animals received fetal bovine lung (FBL) cell culture material that contained  $1.8 \times 10^4$  syncytium forming units of BIV R-29. Because this culture material was contaminated with a non-cytopathic strain of BVDV, all animals were vaccinated with a commercial killed BVDV vaccine 4 and 2 weeks prior to inoculation. Control animals received FBL cells and were infected with a non-cytopathic BVDV. (Field isolate of a non-cytopathic BVDV from persistently infected cattle in Nebraska obtained from S.R. Bolin, NADC, Ames, IA)

#### *Sample collection for Group 2 animals*

Animals were evaluated twice during 1 week at both 4 and 5 months PI for total and differential white blood cell count, neutrophil function, mononuclear cell subset analysis, and lymphocyte blastogenesis.

#### *Group 3: animals 19–27 months PI*

Eight cattle that had been infected with BIV for a period of 19–27 months were used in this experiment. Table 1 indicates the details of the individual animals. The seven (four female, three male) control cattle were 2–5 years of age, and were either Holstein, Holstein/Angus cross-bred, or Charolais/Angus cross-bred. All animals had no detectable antibody to BSV or BLV (agar gel immunodiffusion). Control animals were free of BIV specific antibodies. All BIV infected animals were negative for antibodies to BIV prior to inoculation.

#### *Inocula for Group 3 animals*

The BIV infected animals received different inocula as described in Table 1. The BIV-106 molecular clone (provided by M.A. Gonda, Laboratory of Cell and Molecular Structure, Program Resources, Inc., NCI-Frederick Can-

cer Research Facility, Frederick, MD) of the virus was derived from the original R-29 isolate of BIV (Braun et al., 1988; Garvey et al., 1990). Control animals received no inoculation.

#### *Sample collection for Group 3 animals*

All animals were tested at least twice during a 3 week period for total and differential white blood cell count, neutrophil function, and lymphocyte blastogenesis. Mononuclear cell subset analysis was performed once for each animal.

### *Methods*

#### *In vitro immune function*

Blood (250 ml) was collected by jugular venipuncture into acid citrate/dextrose anticoagulant. After centrifugation, plasma was discarded and the buffy coat was removed for mononuclear cell isolation by density gradient centrifugation, as previously described (Roth et al., 1982). Neutrophils were isolated from the packed red blood cells (RBC), by hypotonic lysis of the RBC as previously described (Roth and Kaeberle, 1981b). Isolated neutrophils were suspended in 0.015 M phosphate-buffered saline solution (PBSS) at a concentration of  $5.0 \times 10^7$  cells  $\text{ml}^{-1}$  for use in the functional assays.

Neutrophil function assays were performed as described previously (Roth and Kaeberle, 1981b; Steinbeck et al., 1986; Chiang et al., 1991). Briefly, random migration under agarose was measured after an incubation period of 18 h; the area of random migration was reported in  $\text{mm}^2$ . Chemotaxis was measured by migration under agarose toward zymosan-activated serum; the chemotactic index was determined by dividing the distance of directed migration by the distance of random migration. Phagocytosis was measured using antibody-coated [ $^{125}\text{I}$ ]iododeoxyuridine-labeled *Staphylococcus aureus*. Neutrophils were incubated for 10 min with bacteria at a ratio of 60:1 (bacteria to neutrophil), then lysostaphin was added to remove the extracellular *S. aureus*; results were reported as percentage of bacteria ingested. Reduction of cytochrome *c*, a measure of superoxide anion production, was evaluated after a 30 min (Group 1) or a 5 min (Groups 2 and 3) incubation of neutrophils with cytochrome *c* and opsonized zymosan. Results were reported as optical density per  $1.25 \times 10^6$  neutrophils per 5 or 30 min. The iodination reaction, a measure of the myeloperoxidase- $\text{H}_2\text{O}_2$ -halide system, was measured by incubating neutrophils with opsonized zymosan and  $\text{Na}^{125}\text{I}$  for 20 min; the reaction was terminated by addition of trichloroacetic acid. Results were reported as  $\text{nmol NaI } 10^{-7}$  neutrophils  $\text{h}^{-1}$ . Antibody-dependent cell-mediated cytotoxicity (ADCC) was evaluated using antibody-coated  $^{51}\text{Cr}$ -labeled chicken RBC as the target cell. The effector-to-target cell ratio was 10:1, and results were reported as percentage of specific release during a 2 h incubation. Antibody-independent cell-mediated cytotoxicity (AICC) was

measured similarly to ADCC; however, antibody was not added. Lymphocyte blastogenesis was performed using pokeweed mitogen, phytohemagglutinin (PHA), and concanavalin A as mitogens, with a 60 h incubation period as described by Roth et al. (1982). Mitogens were used at concentrations that resulted in optimal stimulation of lymphocytes from control cattle.

#### *Antibodies for mononuclear cell subset analysis*

Primary antibodies were purchased from VMRD (Pullman, WA). The hybridoma designation for each antibody follows the cell type the antibody was used to detect (Bo indicates bovine): BoCD4, CACT83B; BoCD8, CACT80C; BoCD2, CH128A; BoCD6, BAQ82A; B-cells, BAQ44A; monocytes, DH59B; null (non-T, non-B, non-monocyte) cells, BAQ4A (Davis et al., 1987, 1988, 1991; Larsen et al., 1990). Murine monoclonal antibodies against non-lymphocyte antigens were used as isotype controls. The fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG-IgM second antibody was obtained from Caltag Laboratories (South San Francisco, CA).

#### *Mononuclear cell subset analysis by flow cytometry*

Isolated PBMC were suspended in PBSS containing 0.5% bovine serum albumin and 0.1%  $\text{NaN}_3$  (PBSS++) at a concentration of  $5 \times 10^6$  cells  $\text{ml}^{-1}$ . Then 200  $\mu\text{l}$  of cells were added to a U-bottom microtiter plate. The plate was then centrifuged for 60 s at  $160 \times g$ . The supernatant was discarded and the cells were resuspended in 50  $\mu\text{l}$  of the appropriate antibody, diluted 1:100 in PBSS++. The plate was incubated for 30 min on ice, then washed three times using PBSS++. A second 30 min incubation was performed in 50  $\mu\text{l}$  of the 1:100 diluted FITC-conjugate. The cells were washed three times, resuspended, and fixed by the addition of 200  $\mu\text{l}$  of a 1:1 mixture of PBSS and 2% paraformaldehyde in water. The plate was stored at 4°C, in the dark, until flow cytometric analysis was performed 1–5 days later.

#### *Flow cytometry*

A Coulter Epics 752 flow cytometer was used for data collection. Forward and right-angle light scatter as well as fluorescein fluorescence were measured. Gating, using forward and right-angle light scatter, was set such that any contaminating RBC or granulocytes were excluded from the analysis. Because of substantial overlap in the monocyte and lymphocyte populations the analysis was performed using the whole mononuclear cell population. Discriminators were set using the second antibody control for each animal so that background fluorescence was always less than 5% (2% for the 4–5 and 19–27 month PI data) of the cells surveyed. For each sample, 5000 cells were analyzed.

### *Hematology*

Blood samples for hematologic evaluation were collected in evacuated blood collection tubes containing sodium EDTA. Hemoglobin concentrations were measured by optical density. Total leukocyte counts were made using an electronic particle counter. Differential leukocyte determinations were made by examining 200 leukocytes on Wright-stained blood smears.

### *Serology and virus isolation*

Western blot analysis and virus isolations by cocultivation with FBL cells were performed as previously reported (Whetstone et al., 1990, 1991). The ELISA titers reported for BIV are from an ELISA that detects antibody to the whole virus (Whetstone, 1992). The virus is grown as previously described (Whetstone et al., 1991). Plates are coated with antigen (virus) in carbonate buffer, pH 9.6, and blocked with 0.1% bovine serum albumin + 0.2% Tween 20. Washes are carried out with 0.02% Tween 20 in 0.01M PBS pH 7.4. Negative control sera were negative at a 1:10 dilution.

### *Statistical analysis*

The neutrophil function, lymphocyte blastogenesis, mononuclear cell subset, and hematology data were analyzed by week of the experiment using a repeated measures analysis of variance as appropriate for the Group 1 data. The data from Group 2 were analyzed using a repeated measures analysis of variance. Similarly, the data from Group 3 were analyzed using a repeated measures analysis of variance including a blocking factor for the date of assay, since all animals were not assayed on the same day. A simple analysis of variance blocking on assay day was used for the mononuclear cell subset data of the 19–27 month PI animals.

## RESULTS

### *Virus recovery and antibody response*

All BIV inoculated animals developed BIV specific antibodies that were detectable by Western blot analysis, except that two of the five inoculated animals in Group 1 did not seroconvert. The data reported for Group 1 is from the three antibody positive BIV inoculated animals. Virus was recovered from one of these three animals in Group 1 at 60 days PI. BIV was reisolated from all animals in Group 2 at both 4 and 6 months PI. ELISA titers to BIV were 1:150 for all four animals in Group 2 at the time of immune function testing. In Group 3 BIV was recovered from three of eight animals. Virus recovery was not attempted on one animal, and recovery was unsuccessful from the other four animals in Group 3 (Table 1). Titers to BIV by ELISA were 1:50 or 1:100 for all Group 3 animals (Table 1).

TABLE 1

Characteristics of the cattle infected with BIV for 19–27 months

Animal	Sex	Breed	BIV inoculation	Age at inoculation	Age at leukocyte evaluation	BIV isolation	BIV ELISA titer
246	M	Jersey	R29 cell culture, IV <sup>1</sup>	10 months	37 months	+	1:50
1029	F	Holstein/Angus	R29 whole blood, fourth passage, IV	5 weeks	20 months	ND	1:100
1034	F	Holstein/Angus	BIV-106 clone cell culture, IV	6 hours	23 months	+	1:50
1049	M	Angus	R29 cell culture, IV	8 months	29 months	–	1:100
1060	M	Holstein/Angus	R29 whole blood, second passage, IV	3 weeks	21 months	+	1:50
1187	F	Holstein	BIV-106 clone cell culture, intracisternal	3 months	26 months	–	1:100
1188	F	Holstein	BIV-106 clone cell culture, intracisternal	3 months	26 months	–	1:50
1476	F	Angus/Hereford	BIV-106 clone cell culture, intracisternal	3 months	26 months	–	1:100

All cattle developed antibodies specific for BIV antigens after inoculation as detected by Western blot analysis. They had no detectable antibodies to either bovine leukemia virus or bovine syncytial virus. <sup>1</sup>IV, intravenous.

### *Clinical signs and hematology*

All animals were clinically normal during the period of evaluation. There were no significant ( $P < 0.05$ ) differences noted in rectal temperatures or other clinical signs between control and BIV infected animals. The only hematologic measure (hemoglobin, total and differential leukocyte count) that was significantly different between control and BIV infected animals was that BIV infected animals at 19–27 months PI had greater total leukocyte counts as a result of an increase in neutrophils ( $P < 0.05$ ; BIV, 3040 neutrophils  $\mu\text{l}^{-1}$ ; control, 1980 neutrophils  $\mu\text{l}^{-1}$ ).

### *Mononuclear cell subset analysis*

Based on the previous reports of lymphoproliferation and alteration in T cell subsets following BIV infection, we were interested in evaluating mononuclear cell subset populations during these experiments. The only difference observed between BIV and control animals was an increase in the percentage of blood mononuclear cells identified as null cells in Group 3 animals (Table 2). Null cell populations at 0–2 and 4–5 months PI were similar in BIV and control groups. The importance of the change in null cell populations is questionable since it only occurred in one group. Table 2 represents 60 comparisons of which one would expect to find three significant at the  $P < 0.05$  level by random chance. There were no differences in the percentages of cells iden-



TABLE 2

Percentage of PBMC for BIV infected and control cattle

Cell	0-2 months PI <sup>1</sup>		4-5 months PI			19-27 months PI		
	BIV ( <i>n</i> =3)	Control ( <i>n</i> =3)	BIV ( <i>n</i> =4)	Control ( <i>n</i> =5)	Pr> <i>F</i>	BIV ( <i>n</i> =8)	Control ( <i>n</i> =7)	Pr> <i>F</i>
BoCD4	24	24	24	24	0.84	25	26	0.93
BoCD8	11	12	12	11	0.49	12	15	0.34
BoCD2/CD6 (Pan T) <sup>2</sup>	33	34	34	33	0.82	36	38	0.77
Null	15	13	16	15	0.75	15	9	0.03
Monocyte	17	16	23	20	0.58	15	14	0.74
B-cell	27	27	20	25	0.16	25	28	0.74

Bo, bovine; Pr>*F*: the probability of having a greater *F* value for the comparison of BIV to control groups simply by chance. *n* is number of animals per group.

<sup>1</sup>The means presented for the 0-2 month data are the mean for each treatment over all assay days. Data were analyzed weekly for 8 weeks post inoculation; hence no Pr>*F* is appropriate for these means. No consistent significant differences were found between BIV and control animals. Occasional differences at *P*<0.05 did occur but the differences were not consistent and occurred at a frequency of less than one in 20 comparisons.

<sup>2</sup>BoCD6 was used for the 0-2 month experiment (CD6 is expressed on most T-cells and on some B-cells). BoCD2 was used for the other two experiments (a Pan T marker).

TABLE 3

Lymphocyte blastogenic response to mitogens for control and BIV infected cattle

Mitogen	0-2 months PI <sup>1</sup>		4-5 months PI			19-27 months PI		
	BIV ( <i>n</i> =3)	Control ( <i>n</i> =3)	BIV ( <i>n</i> =4)	Control ( <i>n</i> =5)	Pr> <i>F</i>	BIV ( <i>n</i> =8)	Control ( <i>n</i> =7)	Pr> <i>F</i>
Phytohemagglutinin ( <i>A</i> counts min <sup>-1</sup> )	45700	29200	17900	8700	0.03	26100	11900	0.06
Concanavalin A ( <i>A</i> counts min <sup>-1</sup> )	21700	19800	17000	9400	0.24	24900	14000	0.15
Pokeweed mitogen ( <i>A</i> counts min <sup>-1</sup> )	19000	29500	5800	2400	0.34	4800	3600	0.18
Unstimulated (counts min <sup>-1</sup> )	700	500	700	700	0.92	600	320	0.11

Pr>*F*: the probability of having a greater *F* value for the comparison of BIV to control groups simply by chance. *A* counts min<sup>-1</sup> is difference in counts min<sup>-1</sup> (counts min<sup>-1</sup> with mitogen - unstimulated counts min<sup>-1</sup>). *n* is number of animals per group.

<sup>1</sup>The means presented for the 0-2 month data are the mean for each treatment over all assay days. Data were analyzed weekly for 8 weeks post inoculation; hence no Pr>*F* is appropriate for these means. No consistent significant differences were found between BIV and control animals. Occasional differences at *P*<0.05 did occur but the differences were not consistent and occurred at a frequency of less than one in 20 comparisons.

TABLE 4

Neutrophil function in BIV infected and control cattle

Test	0-2 months PI <sup>1</sup>		4-5 months PI		Pr>F	19-27 months PI		
	BIV (n=3)	Control (n=3)	BIV (n=4)	Control (n=5)		BIV (n=8)	Control (n=7)	Pr>F
Random migration (mm <sup>2</sup> )	39	42	33	38	0.24	28	33	0.28
Chemotaxis (directed/random)	1.58	1.47	1.48	1.52	0.63	1.54	1.63	0.58
<i>S. aureus</i> ingestion (%)	25	24	36	35	0.63	47	44	0.42
Iodination (nmol NaI 10 <sup>-7</sup> PMN h <sup>-1</sup> )	22	20	33	32	0.52	38	52	0.02
Cytochrome <i>c</i> reduction (OD)	0.65	0.62	0.75	0.82	0.17	0.95	0.96	0.83
AICC (% specific lysis)	5.8	12.6	3.3	6.1	0.03	2.8	3.5	0.69
ADCC (% specific lysis)	48	53	38	53	0.01	38	53	0.02

Pr>F: the probability of having a greater *F* value for the comparison of BIV to control groups simply by chance. *n* is number of animals per group.

<sup>1</sup>The means presented for the 0-2 month data are the mean for each treatment over all assay days. Data were analyzed weekly for 8 weeks post inoculation; hence no Pr>F is appropriate for these means. No consistent significant differences were found between BIV and control animals. Occasional differences at *P*<0.05 did occur but the differences were not consistent and occurred at a frequency of less than one in 20 comparisons.

tified as monocytes, B cells, CD2<sup>+</sup> cells, CD4<sup>+</sup> cells, or CD8<sup>+</sup> cells in any of the groups tested (Table 2). Comparisons of the actual number of cells in each subset yielded similar conclusions.

#### *Lymphocyte blastogenesis and neutrophil function*

The results of the lymphocyte blastogenesis assays indicated a tendency for BIV to increase the blastogenic response to mitogens. In eight of nine comparisons the mean response of BIV infected animals was greater than control animals (Table 3). The response to PHA was significantly higher at 4-5 months PI (*P*<0.05) and nearly so again at 19-27 months PI (*P*<0.06). The response to the other mitogens tested was not significantly altered by BIV infection. Neutrophil ADCC and AICC were both decreased (*P*<0.05) at 4-5 months PI. Iodination and ADCC were also decreased in the BIV infected animals at 19-27 months PI (*P*<0.03). Other measures of neutrophil function were not affected by BIV infection (Table 4).

Because of the nature of lentiviral infections, the disease course may be expected to differ in different animals. Therefore, in addition to the statistical analyses comparing groups of animals, the raw data were examined to deter-

mine if any individual animal or subset of animals deviated notably from normal in any of the various measures. None of the individual animals had any measures which markedly and consistently varied from normal. We also found no differences in Groups 1 and 3 when comparing animals that virus had been recovered from, with those that virus had not been recovered from.

## DISCUSSION

Taken collectively, these data indicate that BIV infection is capable of inducing alterations in the bovine immune system. Initially, no alterations were observed in animals tested three times a week for the first 2 months following infection. However, at 4–5 months PI neutrophil ADCC and AICC were significantly decreased while lymphocyte blastogenesis to PHA was increased. At 19–27 months PI, neutrophil iodination, a measure of degranulation and oxidative metabolism, was decreased in addition to the decrease in ADCC. Lymphocyte blastogenesis to PHA was higher in BIV infected animals 19–27 months PI. The fact that decreased ADCC and increased blastogenesis to PHA were recorded in two separate groups of BIV infected animals adds to the significance of these observations. The increase in blastogenesis to PHA is consistent with the previous observations that BIV infection causes lymphoid proliferation rather than lymphoid depletion in infected cattle (Van der Maaten et al., 1972; Carpenter et al., 1992).

Our observations differ from the previously reported decreases in lymphocyte blastogenesis (Terpstra et al., 1989) and in CD4 cell numbers (Stott et al., 1989) following BIV infection. Differences in inocula, concurrent infections, and individual animal variation may have contributed to the contrasting observations. The reported decrease in lymphocyte blastogenesis was observed at 6 months PI in two animals that were tested 2 and 6 months PI (Terpstra et al., 1989). We observed an increase in lymphocyte blastogenesis at 4–5 months PI and 19–27 months PI. The previously reported decrease in CD4 cells occurred at 3–4 months after infection (Stott et al., 1989). Similar to our experiment, the calves in the previous reports were challenged with high passage BIV R-29 infected cell culture material that contained BVDV. Lymphoid depletion has been reported to follow lymphoproliferation in HIV and simian immunodeficiency virus infection (Chalifoux et al., 1987; Gerstoft et al., 1989; Ost et al., 1989). The decreased blastogenesis and drop in CD4<sup>+</sup> cells may be a transient event which we did not detect.

The R-29 isolate is the only reported isolate of BIV. However, this isolate of BIV had been passed multiple times in tissue culture, and it may have become tissue culture adapted, losing some of its original virulence. In addition, all available cultures of the R-29 virus were also co-infected with a non-cytopathic strain of BVDV. The BVDV is known to induce neutropenia and lymphopenia, and to impair neutrophil and lymphocyte function during the first

month after infection (Tyler and Ramsay, 1965; Malmquist, 1968; Roth et al., 1981, 1986). In an attempt to increase the virulence and to obtain a challenge inoculum which was free of BVDV the R-29 isolate was serially passed through calves. The serial passage did succeed in eliminating the co-infecting BVDV, but apparently did not restore the R-29 isolate to its original virulence (Carpenter et al., 1992). The inoculum given to the Group 1 animals appears to have been a minimal dose for BIV infection: only three of five seroconverted and virus was recovered from only one animal. Possibly, some virus viability was lost during the preparation and storage of the frozen inoculum used for this experiment. The lack of any immune function alterations observed in this group may have been the result of the low virulence of the inoculum rather than the inability of BIV to cause such changes. A different method for preparing the inoculum for the Group 2 animals was used in an attempt to establish a more consistent infection. Immune function was not evaluated in Group 2 animals until 4 months after infection to ensure that the BVDV present in the inoculum had been cleared by the animals. The R-29 virus, grown to a high titer in FBL cell cultures, proved to be a good challenge inoculum since all Group 2 animals produced antibody, and BIV could be successfully recovered from them.

BIV infection did not cause any clinical signs of disease in these animals. Therefore, the biological significance of the recorded alterations in immune function are not clear. Yet, similar decreases in ADCC and iodination have been observed in BVDV infected cattle and cattle treated with dexamethasone (Roth and Kaerberle, 1981a, 1984, 1985; Roth et al., 1981, 1986; Roth and Frank, 1989). Both BVDV infection and dexamethasone treatment have been associated with increased disease incidence. Neutrophil ADCC is thought to be an important antiviral mechanism for cattle. The myeloperoxidase-H<sub>2</sub>O<sub>2</sub>-halide system, measured by the iodination assay, is a potent bacteriocidal mechanism of the neutrophil. A defect in this system may lead to increased susceptibility to bacterial infection. The animals in this study were housed in isolated groups and were not exposed to animals with clinically significant bacterial infections.

The effect of BIV may become more marked as the time after infection increases. This pattern would be similar to other mammalian lentiviruses. Recent studies have indicated changes in T-cell subsets were detectable in feline immunodeficiency virus infected cats at 6 months post infection; deficits in proliferative responses to mitogens were progressive beginning at 6 months post infection (Torten et al., 1991). During HIV infection, changes in T-cell subsets are evident by 6 months after the first detection of serum antibody to HIV (Giorgi and Detels, 1989); however, clinical disease is often not apparent for years. Ovine lentivirus infection usually occurs by colostral transmission shortly after birth, but the pneumonia associated with Maedi/Visna is usually not observed until the animal is 2-3 years of age (Cutlip et

al., 1988). In a like manner, the disease associated with caprine arthritis-encephalitis virus may take months or years to develop (Cheevers and McGuire, 1988). In contrast, the equine infectious anemia virus may induce clinical disease within several days after infection (Cheevers and McGuire, 1988).

BIV infection does appear to cause alterations in bovine lymphocyte and neutrophil function by 4–5 months after infection. Current inocula do not cause the degree of lymphocytosis induced by the original isolate. Following infected animals for a longer time after infection and work with additional isolates are needed to further define the potential significance of BIV infection on the bovine immune system.

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