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Effect of infection with bovine leukosis virus on lymphocyte proliferation and apoptosis in dairy cattle

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Objective—To determine effects of infection with bovine leukosis virus (BLV) on lymphocyte proliferation and apoptosis in dairy cattle.

Animals—27 adult Holstein cows.

Procedures—Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood from lactating Holstein cows seronegative for BLV (n = 9 cows), seropositive for BLV and aleukemic (aleukemic; 9), and seropositive for BLV and persistently lymphocytotic (PL; 9). Isolated PBMCs were assayed for mitogen-induced proliferation and were analyzed by means of flow cytometry. The PBMCs from a subset of each group were assayed for apoptosis, caspase-9 activity, and expression of selected genes related to apoptosis.

Results—PL cows had significantly higher total lymphocyte counts and significantly lower proportions of T-lymphocyte populations than did BLV-negative and aleukemic cows. Both groups of BLV-infected cows had significantly higher proportions of B cells and major histocompatibility complex II–expressing cells than did BLV-negative cows. Proliferation with concanavalin A was significantly lower for PL cows, compared with proliferation for BLV-negative cows. Pokeweed mitogen–induced proliferation was significantly higher for aleukemic and PL cows than for BLV-negative cows. Gene expression of apoptosis-inhibitory proteins BCL2 and BCL2L1 was significantly higher for aleukemic cows and expression of BCL2 was significantly higher for PL cows than for BLV-negative cows.

Conclusions and Clinical Relevance—Cattle infected with BLV had marked changes in PBMC populations accompanied by alterations in proliferation and apoptosis mechanisms. Because the relative distribution and function of lymphocyte populations are critical for immune competence, additional studies are needed to investigate the ability of BLV-infected cattle to respond to infectious challenge. (*Am J Vet Res* 2011;72:1059–1064)

Enzootic bovine leukosis is a contagious disease of cattle induced by an exogenous retrovirus, BLV. The disease complex is characterized by persistent lymphocytosis, which can culminate in lymphoma.[1] Bovine leukemia virus is present in 89% of US dairy operations.[2] Mean losses are estimated to be $59/cow for herds seropositive for BLV, with losses to the dairy industry of $285 million for producers and $240 million for consumers.[3] Most infected cows do not have signs of disease, and these cattle are referred to as subclinical or aleukemic. Approximately 30% to 40% of cattle that are carriers of BLV will develop persistent lymphocytosis, but < 5% develop malignant lymphosarcoma.[1] However, malignant lymphosarcoma is the largest single reason for cows (beef and dairy) to be condemned during postmortem inspection at slaughter plants and accounts for > 21% of all condemnations.[4]

Abbreviations

7-AAD	7-aminoactinomycin D
BLV	Bovine leukemia virus
IL	Interleukin
PBMC	Peripheral blood mononuclear cell
PL	Persistently lymphocytotic
PWM	Pokeweed mitogen

The progression of BLV substantially affects host defense mechanisms. Although BLV is associated primarily with infections of B lymphocytes, BLV provirus has been detected in the DNA of immunoaffinity-purified T lymphocytes from BLV-infected cattle.[5] There is a dramatic increase in B-lymphocyte populations with significant decreases in the percentages of $CD4^+$ and $CD8^+$ T-lymphocyte populations.[6] Concentrations of certain type 1 cytokines (including IL-2, IL-12, and interferon-γ) from $CD4^+$ T lymphocytes are reduced during BLV infections, and it has been suggested that this altered cytokine production is responsible for suppressed mitogen-induced T-lymphocyte proliferation.[6–8] Examination of cytokine

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profiles from all PBMC populations, including B lymphocytes, suggests that type I and II cytokines are altered with increases in IL-10 and IL-4 concentrations and decreases in concentrations of IL-2, IL-12, and interferon-γ.[7,9]

The protracted persistence of B lymphocytes in the bloodstream is thought to reflect the onset of uncontrolled proliferation, reduced apoptotic processes, or both. In PL cattle, a decrease in the turnover of B lymphocytes is associated with a reduced rate of cell death.[10] It has also been suggested that increased expression of tumor necrosis factor and changes in expression of tumor necrosis factor receptors enhance B-lymphocyte proliferative responses in PL cattle.[7,11] Thus, the development of leukosis is a complex process that involves altered regulation of cell proliferation and apoptosis of the immune cell populations of cattle. The purpose of the study reported here was to evaluate the effect of stage of BLV infection on changes in proliferation and apoptosis in specific lymphocyte subsets and concurrent expression of genes associated with the rate of cell turnover.

Materials and Methods

Animals—Twenty-seven adult (≥ 2 lactations) lactating (> 60 days of lactation) Holstein cows were assigned to 1 of 3 groups. Cows were classified as BLV-negative (n = 9) if 2 serum samples, obtained 3 months apart, yielded negative results for anti-BLV antibodies (ie, seronegative on the basis of results of an ELISA) and leukograms (based on total WBC counts and differential cell counts for whole blood samples) were within laboratory reference ranges used for cattle, as established by the participating diagnostic laboratory. Cows were designated as BLV-positive aleukemic (n = 9) if both serum samples yielded positive results for BLV (ie, cows were seropositive) and the total lymphocyte count in whole blood samples was < 7,500 cells/μL. Cows were designated as PL (n = 9) if both serum samples yielded positive results for BLV (ie, cows were seropositive) and the total lymphocyte count in whole blood samples was ≥ 7,500 cells/μL. Cows with clinical signs of disease (eg, mastitis, lameness, and chronic weight loss) were excluded from the study. The study protocol was approved by the Michigan State University Institutional Animal Use and Care Committee.

BLV ELISA and hematologic analysis—Blood samples for testing with an ELISA were collected from the coccygeal vein into a 15-mL evacuated tube,[a] allowed to clot overnight at 4°C, and centrifuged at 2,400 × *g* for 30 minutes at 4°C prior to serum harvest. Additionally, blood samples were collected into a 7-mL sterile glass tube that contained EDTA; these samples were used to determine the total and differential leukocyte concentration. Hematologic analysis for leukocyte concentrations and use of the ELISA to determine BLV infection status were performed at the Diagnostic Center for Population and Animal Health at Michigan State University.

Isolation of PBMCs—A large volume of whole blood was necessary to ensure an adequate number of PBMCs for immune assays. Therefore, an additional 50 mL for use in cell isolation was collected from a jugular vein into tubes that contained sodium citrate as an anticoagulant. Briefly, the blood was centrifuged at 931 × *g* for 30 minutes at 15°C; a 5-mL aliquot of the buffy coat was aspirated and transferred into a 50-mL centrifuge tube that contained 20 mL of Hank's buffered salt solution. The cell suspension was then layered over 12.5 mL of sterile density-gradient centrifugation medium[b] in a 50-mL centrifuge tube and centrifuged for 30 minutes at 424 × *g* at 20°C. The mononuclear cell layer was aspirated, added to a tube that contained 20 mL of Hank's buffered salt solution, and centrifuged for 10 minutes at 233 × *g* at 15°C. Red blood cells were lysed by incubation with 10 mL of double-distilled water for 18 seconds and then neutralized by the addition of 10 mL of 2× RPMI medium.[c] Cells were washed twice with Hank's buffered salt solution by centrifuging for 10 minutes at 233 × *g* at 4°C before viable cells were counted by means of trypan blue exclusion.

Flow cytometric analysis—To phenotypically characterize isolated PBMCs, flow cytometric analysis was performed via incubations with monoclonal antibodies specific for bovine leukocyte antigens. Cells (1×10^6) were incubated with each of the following lineage-specific bovine monoclonal antibodies: CD3[d] (T-cell receptor; dilution of 1:100 [vol/vol]), CD4[e] (T-helper; dilution of 1:160 [vol/vol]), CD8[f] (T-cytotoxic and suppressor; dilution of 1:400 [vol/vol]), B-B2[g] (B lymphocytes; dilution of 1:150 [vol/vol]), monocyte and granulocyte[h] (dilution of 1:100 [vol/vol]), and major histocompatibility complex II receptor[i] (dilution of 1:200 [vol/vol]). An irrelevant antigen-control CD18[j] (dilution of 1:100 [vol/vol]) was assayed in parallel to detect nonspecific labeling because of Fc-receptor binding. Cell surface markers were stained by use of goat anti-mouse $F(ab)_2$ IgG–fluorescein isothiocyanate[k] via a staining procedure described elsewhere.[12] Mononuclear cells were gated by means of forward and side-light scatter, and data were collected for 10,000 events by use of a flow cytometer.[l] After subtracting values for the control samples, the percentage of immunofluorescence was expressed as the number of positive cells divided by the number of total cells, with the quotient multiplied by 100.

Proliferation assay—The proliferative response of PBMCs was determined after they were incubated with various mitogens. Briefly, PBMCs (1×10^5) were seeded in white-walled 96-well plates without mitogen (control cells) or with concanavalin A (5 μg/mL) or PWM (1 μg/mL). After PBMCs were incubated for 48 hours at 37°C, a commercial assay[m] was used to measure proliferation on the basis of quantifying ATP. As described in the assay protocol, the prepared substrate was added to the cells to promote cell lysis and generate a luminescent signal proportional to the amount of ATP. Relative luminescence was detected by use of a multilabel plate reader.[n] A stimulation index was calculated as the relative luminescence of mitogen-stimulated cells divided by the luminescence for unstimulated (control) cells within each BLV-infected group.

Real-time PCR assay—Separate samples of whole blood were collected and placed into tubes that contained EDTA; these samples were used to assay for ex-

pression of 3 genes associated with cell apoptosis: BAX, BCL2, and BCL2L1. Total mRNA was extracted from whole blood obtained from BLV-negative (n = 3), aleukemic (6), and PL (5) cows by use of an RNA blood kit.[o] An on-column DNase digest was performed in conjunction with the RNA blood kit[o] to remove genomic DNA. Purified RNA was converted to cDNA by use of a high-capacity cDNA archive kit.[p] Real-time PCR assay was performed by use of a real-time PCR system[q] that involved the use of custom-designed gene expression assays[r] with minor groove–binding probes. Polymerase chain reactions were performed in triplicate by use of 20 µL of reaction mixture/well, which consisted of 10 µL of 2X premixed solution,[s] 1 µL of a custom 20X gene expression assay mix,[t] 50 ng of cDNA, and nuclease-free water. Each gene (BAX, BCL2, and BCL2L1) was individually amplified with the same reaction mixture. A custom 20X gene expression assay mix[t] for bovine β-actin was used as an endogenous control sample. Thermal cycling conditions for fast 2-step PCR assays were used (enzyme activation at 95°C for 20 seconds followed by 40 repetitions of 95°C for 3 seconds and 60°C for 30 seconds). Quantification was performed with the relative quantification method. The amount of target, normalized on the basis of β-actin and relative to a calibrator, was calculated as $2^{-\Delta\Delta Ct}$, where Ct is the cycle number at which the fluorescence signal of the product crosses an arbitrary threshold set with exponential phase of the PCR assay and ΔΔCt is calculated as (Ct target of unknown sample – Ct β-actin of unknown sample) – (Ct target of calibrator sample – Ct β-actin of calibrator sample), as described elsewhere.[13]

Caspase-9 activity assay—The activity of caspase-9 in PBMCs was measured by use of a commercial assay kit.[u] Briefly, PBMCs (5×10^4) from 12 cows (4 cows/BLV-infected group) were seeded in white-walled, 96-well plates. Cells were incubated for 24 hours at 37°C, and caspase-9 reagent[u] was prepared in accordance with the assay protocol and added to the cells to initiate cell lysis. By use of a multilabel plate reader,[n] caspase cleavage of the substrate and subsequent generation of a luminescent signal proportional to the amount of caspase activity were reported as the number of relative luminescence units.

Apoptosis of lymphocyte subsets—The amount of apoptosis in T cells and B cells was determined by use of flow cytometric techniques and a commercial apoptosis assay kit.[v] Briefly, PBMCs from 9 cows (3 cows/BLV-infected group) were incubated with monoclonal antibodies specific for CD3[d] (T-cell receptor; dilution of 1:100 [vol/vol]) or B-B2[g] (B lymphocytes; dilution of 1:100 [vol/vol]) and then stained with goat anti-mouse $F(ab)_2$ IgG–fluorescein isothiocyanate. After the final wash step, PBMCs were resuspended in 100 µL of 1X annexin V binding buffer, which was followed by the addition of 5 µL of annexin V[w] and 0.5 µL of 7-AAD (1 mg/mL). Samples were gently mixed and incubated for 30 minutes at 22°C; samples subsequently were transferred to tubes that contained an additional 300 µL of 1X annexin V binding buffer and then placed on ice. Samples were analyzed by use of a flow cytometer[l] within 30 minutes after being placed on ice. Mononuclear cells were gated by use of forward and side light scatter, and data were collected for 20,000 events within that gate. From that first gate, the $CD3^+$ or B^+ cells were selected via a second gate. Apoptosis within the lymphocyte subsets was determined by plotting the events within the second gate in a histogram. Live cells had negative results for annexin V and 7-AAD, early apoptotic cells had positive results for annexin V and negative results for 7-AAD, and late apoptotic and dead cells had negative results for annexin V and positive results for 7-AAD. Percentage of immunofluorescence was expressed as the number of positive cells divided by the number of total cells, with the quotient multiplied by 100.

Statistical analysis—Differences between BLV-infected groups were analyzed with statistical software.[x,y] Comparisons of means were made by use of an ANOVA followed by a Tukey-Kramer honestly significant difference post hoc test, as necessary. Data were tested for normality by use of the Kolmogorov-Smirnov method and transformed when necessary. Data were reported as mean ± SEM. Values of $P < 0.05$ were considered significant.

Results

Mean ± SEM total leukocyte counts on the day of blood collection for immune assays in the BLV-negative and aleukemic cows were $7.6 \times 10^3 \pm 0.6 \times 10^3$ cells/µL and $10.1 \times 10^3 \pm 0.9 \times 10^3$ cells/µL, respectively. These counts were significantly lower, compared with those for PL cows ($28.1 \times 10^3 \pm 7.3 \times 10^3$ cells/µL). Similarly, PL cows ($23.2 \times 10^3 \pm 7.2 \times 10^3$ cells/µL) had significantly higher mean total lymphocyte counts than did the BLV-negative ($3.7 \times 10^3 \pm 0.4 \times 10^3$ cells/µL) and aleukemic ($4.7 \times 10^3 \pm 0.6 \times 10^3$ cells/µL) cows. Aleukemic and PL cows had significantly higher proportions of B cells and major histocompatibility complex II–expressing cells, compared with results for BLV-negative cows. The PL cows had significantly lower propor-

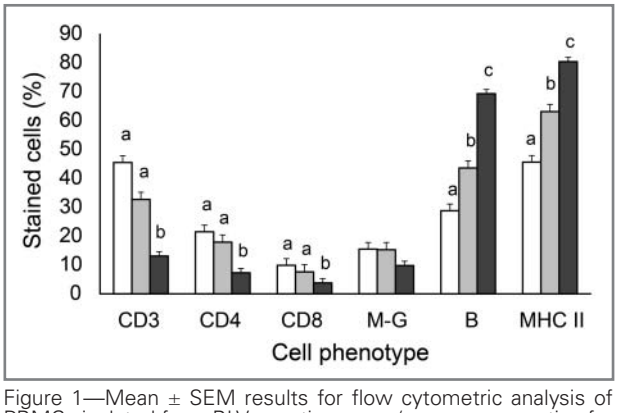

Figure 1—Mean ± SEM results for flow cytometric analysis of PBMCs isolated from BLV-negative cows (cows seronegative for BLV; white bars [n = 9]), BLV-positive (seropositive for BLV) and aleukemic cows (light gray bars [9]), or BLV-positive and PL cows (dark gray bars [9]) and that were stained with specific monoclonal antibodies. The PBMCs were isolated and seeded at 1×10^6 cells/well on a 96-well plate prior to staining. B = B lymphocytes. M-G = Monocyte and granulocyte. MHC II = Major histocompatibility complex II. [a–c] Within each cell phenotype, values with different superscript letters differ significantly ($P < 0.05$) among BLV-infected groups.

tions of $CD3^+$, $CD4^+$, and $CD8^+$ cells, compared with results for BLV-negative and aleukemic cows (**Figure 1**).

Proliferation, as measured via increased cell-induced luminescence in media without mitogen, was significantly higher in PL cows than in aleukemic or BLV-negative cows (data not shown). Concanavalin A and PWM induced significant increases in PBMC proliferation, relative to that in unstimulated control cells, for all 3 BLV-infected groups (data not shown). Concanavalin A–induced proliferation was significantly higher in BLV-negative cows than in PL cows. However, PWM-induced proliferation was significantly higher in aleukemic and PL cows than in BLV-negative cows (**Figure 2**). Caspase-9 activity or gene expression of the apoptosis promoter BAX did not differ significantly among the BLV-infected groups (**Figures 3 and 4**). However, gene expression of the apoptosis inhibitory protein BCL2 was significantly higher in aleukemic and PL cows than in BLV-negative cows. Similarly, gene expression of the apoptosis inhibitory protein BCL2L1 was significantly higher in aleukemic cows than in BLV-negative and PL cows. The combined proportion of apoptotic and dead cells for the T-lymphocyte subset was significantly lower in aleukemic cows, compared with results for the PL cows (**Figure 5**). The proportion of apoptotic and dead

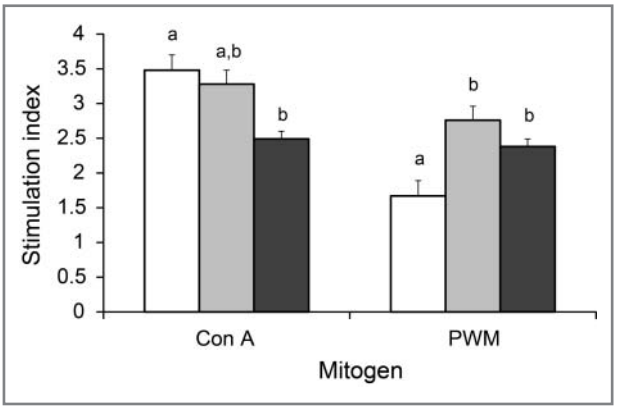

Figure 2—Mean ± SEM lymphoproliferative response to mitogens for PBMCs isolated from BLV-negative cows (white bars [n = 9]), BLV-positive aleukemic cows, (light gray bars [9]), and BLV-positive PL cows (dark gray bars [9]). The PBMCs were isolated and seeded at 1×10^5 cells/well in a 96-well plate; cells were incubated for 48 hours at 37°C without a mitogen (control cells) or with concanavalin A (Con A; 5 µg/mL) or PWM (1 µg/mL). The stimulation index (calculated as the number of cells in mitogen-treated wells/number of cells in control wells) was determined within each BLV-infected group. [a,b] Within each mitogen treatment, values with different superscript letters differ significantly ($P < 0.05$) among BLV-infected groups.

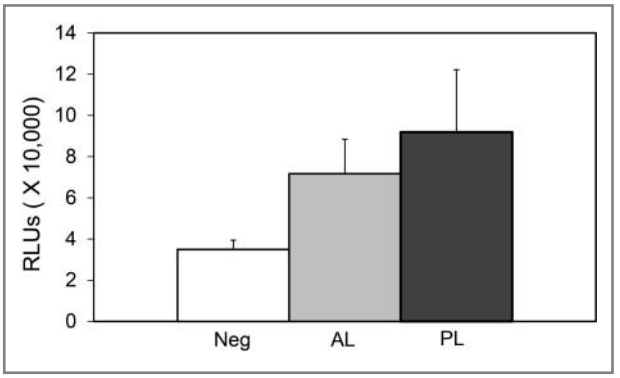

Figure 3—Mean ± SEM caspase-9 activity of PBMCs isolated from BLV-negative cows (Neg; white bar [n = 4]), BLV-positive aleukemic cows (AL; light gray bar [4]), and BLV-positive PL cows (PL; dark gray bar [4]). The PBMCs were isolated and seeded at 5×10^4 cells/well in a 96-well plate; cells were incubated for 24 hours at 37°C. Values did not differ significantly ($P > 0.05$) among BLV-infected groups. RLUs = Relative luminescence units.

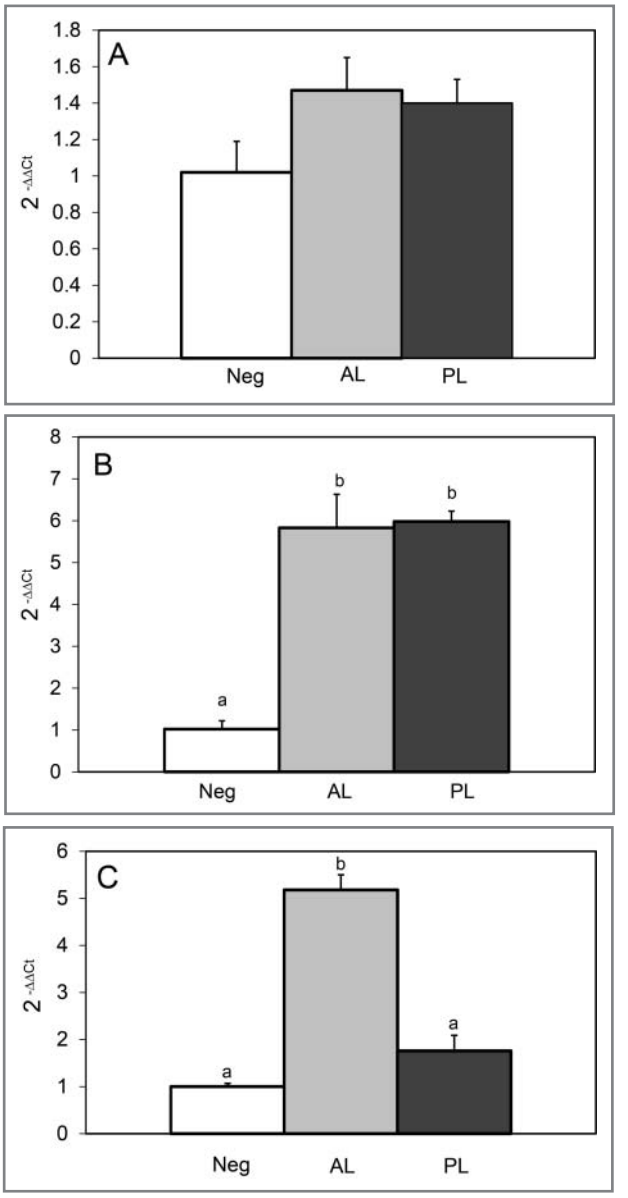

Figure 4—Gene expression of BAX (A), BCL2 (B), and BCL2L1 (C) for PBMCs isolated from BLV-negative cows (Neg; white bar [n = 3]), BLV-positive aleukemic cows (AL; light gray bar [6]), and BLV-positive PL cows (PL; dark gray bar [5]). Messenger RNA was extracted from isolated PBMCs, and cDNA produced by use of reverse transcriptase was amplified and measured by use of a real-time PCR assay. Results represent the $2^{-\Delta\Delta Ct}$ values, where Ct is the cycle number at which the fluorescence signal of the product crosses an arbitrary threshold set with the exponential phase of the PCR assay and ΔΔCt is calculated as (Ct target of unknown sample – Ct β-actin of unknown sample) – (Ct target of calibrator sample – Ct β-actin of calibrator sample), with the samples from BLV-negative cows as the calibrator. Notice that the scale of the y-axis differs among panels. [a,b] In each panel, values with different superscript letters differ significantly ($P < 0.05$) among BLV-infected groups.

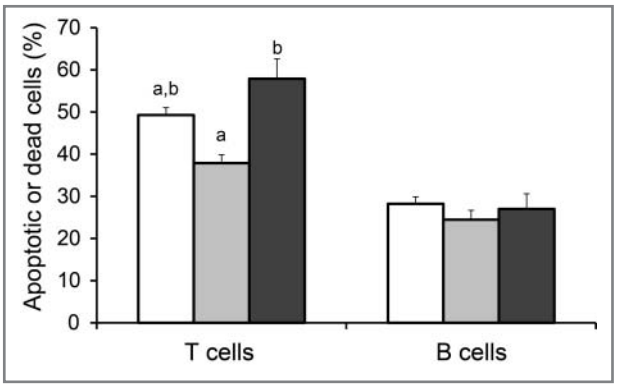

Figure 5—Mean ± SEM percentage of apoptotic or dead cells (stained with annexin or 7-AAD) in lymphocyte subsets for PBMCs isolated from BLV-negative cows (white bars [n = 3]), BLV-positive aleukemic cows (light gray bars [3]), and BLV-positive PL cows (dark gray bars [3]). The PBMCs were isolated and seeded at 1×10^6 cells/well in a 96-well plate; cells were stained with specific monoclonal antibodies to identify lymphocyte subsets. Cells then were incubated with annexin-V and 7-AAD. [a,b]In each lymphocyte subset, values with different superscript letters differ significantly ($P < 0.05$) among BLV-infected groups.

cells in the B-lymphocyte subset did not differ significantly among BLV-infected groups.

Discussion

The classification of PL versus aleukemic cows among BLV-infected cows was performed on the basis of evaluation of the distributions for lymphocyte counts (≥ 7,500 cells/µL) in a population of cattle.[14] However, lymphocyte counts are a continuous variable, and defining an arbitrary limit on BLV status may not allow for optimum discrimination of immunologic responses between these 2 infection groups. In a recent study,[15] investigators found that leukogram reference ranges commonly used by diagnostic laboratories may overestimate the proportion of lymphocytes in dairy cattle. This may have resulted from the inclusion of BLV-infected cattle in previous studies. Thus, reference leukograms should be modified to account for BLV infection. Lymphocyte counts in the PL cows in the study reported here ranged from 10,400 to 79,700 cells/µL; these counts (as well as the mean count for PL cows in the present study) exceeded those in other studies.[6,10] Thus, the results obtained in the present study may indicate a broader spectrum of BLV-infected cattle than previously reported. Additionally, PL cows in the present study may have represented a population with more progression of the disease. Other investigators have determined that in utero or periparturient infection with BLV is more likely in calves whose dams had peripheral blood lymphocyte counts > 12,000 cells/µL.[16] Flow cytometric analysis indicated that PL cows in the present study had a lower proportion of T cells than did cows from the other groups, and aleukemic and PL cows had higher proportions of B cells within the lymphocyte population. These data, in addition to the peripheral lymphocyte counts, are in agreement with results of other reports[1,2,6,10,17] that indicated BLV induces an accumulation of B lymphocytes in the blood and lymphoid tissue as well as potentially causing leukemia, especially in PL cattle.

The progression of BLV disrupts the homeostasis of lymphocyte proliferation and cell death in B cells and T cells.[6,10,11,17] Lymphocytes respond to mitogenic stimulation by producing cytokines, expressing cytokine receptors, and ultimately proliferating. We selected the mitogens for the proliferation assays to try to discriminate responses among lymphocyte subsets. Proliferation in response to concanavalin A is a property of thymus-derived lymphocytes, and responsiveness to PWM is primarily a property of B lymphocytes. The PL cows had lower proliferation in response to concanavalin A than did BLV-negative cows, and both groups of BLV-infected cows had increased lymphocyte proliferation in response to PWM, relative to unstimulated cells. Thus, changes in response to mitogens in lymphocytes from BLV-infected cows may not be limited to B cells alone. Therefore, the present study confirmed that altered proliferation was associated with BLV infection. This is in agreement with results of several other studies.[5,6,8,9,11] In contrast, investigators in 1 study[10] reported that B cells from PL cows have decreased proliferation relative to B cells from aleukemic and BLV-negative cows; effects on T-cell proliferation were not reported. That study[10] differed from most studies because an in vivo method of DNA uptake of a thymidine analogue, rather than mitogen stimulation, was used to measure proliferation. Additionally, PBMCs isolated from PL cows in the present study had higher spontaneous proliferation than did cells isolated from the other BLV-infected groups, even in the absence of mitogen.

Programmed cell death (ie, apoptosis) is a process critical for maintaining normal immune function. Disruption in the rates of cell deaths may be an important dynamic when considering the impact of BLV on the homeostasis of lymphocyte populations.[10,17,18] Analysis of the data reported here suggested that PBMCs from PL cows had increased apoptosis and cell death among T cells, compared with results for PBMCs from aleukemic cows. However, there was no difference in the rate of cell death among B cells, relative to that for the BLV-infected group. This is contrary to results of another report[10] in which investigators found that PL cows had decreased apoptosis in B cells. Investigators in that study[10] used an in vivo method, whereas data for cells undergoing apoptosis and dead cells were included for the study reported here. However, in the present study, expression of the gene for the apoptotic receptor antagonist BCL2 was higher in aleukemic and PL cows; BCL2L1 expression was also higher in the aleukemic cows. In the intrinsic pathway of apoptosis, release of cytochrome c from mitochondria triggers the activation of caspase-9, which in turn activates numerous other caspases (the caspases are a family of proteases that cleave proteins necessary for the integrity of cells). The release of cytochrome c is inhibited by BCL2 and BCL2L1.[18] Increased expression of the BCL2 gene has been identified in invasive B-cell lymphomas because of increased resistance to apoptosis.[18] However, there was no difference in gene expression for the proapoptotic protein BAX. Additionally, caspase-9 activity did not differ in PBMCs from any of the BLV-infected groups. We assayed caspase-9 activity in total PBMC populations and not in lymphocyte subsets, as was performed

for the apoptosis assay. On the basis of previous results and the desire to perform assays contemporaneously, we randomly selected samples from subsets of all cows in the study reported here for use in the assays related to apoptosis. One of the PL cows selected for analysis of gene expression was excluded because of blood clots in the samples.

The balance between cell proliferation and cell death is complex. There are numerous other pathways and proteins identified that were not included in the present study. Additionally, we did not establish results that would account for the interaction between all members of the apoptosis cascade. However, as indicated by the shift in the distribution of PBMCs, our data confirmed that infection with BLV causes a shift in the balance of cell proliferation and cell death. Disruption of this balance could have a detrimental impact on the ability of cattle to resist the development of infectious disease or respond to challenge exposure after vaccination.[5] There is a paucity of studies conducted to investigate this concept, but a clinical observation[19] suggested a possible impairment of rotaviral immune responses in BLV-positive animals. Additionally, a recent but preliminary investigation[20] revealed that immunization with J5 *Escherichia coli* bacterin induced a higher serum IgG2 anti-J5 *E coli* antibody response in BLV-negative dairy cows, as compared with the response in BLV-positive dairy cows.

Cattle persistently infected with BLV have marked changes in total peripheral lymphocytes and the distribution of lymphocyte populations. These changes likely were a result of viral-induced alterations in cell proliferation and apoptosis. The present study revealed that changes in lymphocyte proliferation or apoptosis will depend on the stage of BLV infection. Bovine leukemia virus–induced effects on the complex balance of cell proliferation and programmed cell death are not fully understood. Because lymphoproliferative responses are critical for immune competence, additional studies are needed to investigate the ability of BLV-infected cattle to respond to infectious challenge.

a. Vacutainer, BD Diagnostics, Franklin Lakes, NJ.
b. Ficoll-paque PLUS, GE Life Sciences, Piscataway, NJ.
c. Sigma Chemical Co, St Louis, Mo.
d. MM1A, VMRD Inc, Pullman, Wash.
e. CACT83B, VMRD Inc, Pullman, Wash.
f. CACT80C, VMRD Inc, Pullman, Wash.
g. BAQ44A, VMRD Inc, Pullman, Wash.
h. DH59B, VMRD Inc, Pullman, Wash.
i. TH14B, VMRD Inc, Pullman, Wash.
j. H20A, VMRD Inc, Pullman, Wash.
k. US Biochemical Corp, Cleveland, Ohio.
l. LSR II flow cytometer, Becton-Dickinson, Franklin Lakes, NJ.
m. Promega Cell Titer-Glo luminescent cell viability assay, Promega Inc, Madison, Wis.
n. Wallac VICTOR plate reader, Perkin-Elmer, Waltham, Mass.
o. QIAamp RNA blood kit, Qiagen, Valencia, Calif.
p. Applied Biosystems, Foster City, Calif.
q. 7500 fast real-time PCR system, Promega Inc, Madison, Wis.
r. TaqMan gene expression assays, Applied Biosystems, Foster City, Calif.
s. TaqMan fast universal PCR master mix, Applied Biosystems, Foster City, Calif.
t. Custom TaqMan gene expression assay mix, Applied Biosystems, Foster City, Calif.
u. Promega Caspase-Glo 9 assay, Promega Inc, Madison, Wis.
v. Vybrant apoptosis assay kit No. 14, Molecular Probes Inc, Eugene, Ore.
w. Pacific Blue annexin V, Invitrogen, Carlsbad, Calif.
x. PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.
y. GraphPad Prism, version 4, GraphPad Software Inc, La Jolla, Calif.

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