Glucocorticoid Effects on Peripheral Blood Lymphocytes in Cows Infected With Bovine Leukemia Virus

By John C. Bloom, Simon J. Kenyon, and Thomas G. Gabuzda

We examined the effects of glucocorticoids on peripheral blood lymphocytes (PBL) in lymphoproliferative conditions associated with bovine leukemia virus (BLV): persistent lymphocytosis (PL) and lymphosarcoma cell leukemia (BLSL). The effects of hydrocortisone 21-sodium succinate (HSS) on spontaneous incorporation (SI) and mitogen-stimulated incorporation of radiolabeled-thymidine and the effects of intramuscular administration of prednisolone acetate were studied. An expanded population of B lymphocytes in cows with PL was remarkably sensitive to glucocorticoids in vitro and in vivo. SI was markedly inhibited by concentrations of HSS as low as $10^{-7} \text{M}$. These results correlated well with in vivo observations, where an 80%–90% decrease in PBL occurred during the course of glucocorticoid administration. The decrease in total lymphocytes was accounted for almost entirely by a decrease in the expanded B lymphocyte population. Steroid-sensitive lymphocytes together with steroid-resistant cells were observed in cows with BLSL. The reduction in the steroid-sensitive lymphocytes was associated with rapid disease progression in cows with lymphosarcoma. Steroid-sensitive lymphocyte populations in cows with BLSL may include the same reactive B-cell population found in cows with PL. Glucocorticoids may prove to be a useful tool for study of the immune response to the oncogenic virus and lymphoma in BLV-infected cattle.

BOVINE LEUKEMIA VIRUS (BLV) is a leukemogenic type-C retrovirus that naturally infects cattle. This infection is associated with both persistent lymphocytosis (PL), a benign lymphoproliferative condition, and lymphosarcoma, a systemic malignancy of the lymphoreticular tissue. Although cows with PL are clearly predisposed to develop lymphosarcoma, most do not make this transition. About two-thirds of the lymphosarcomatous animals have a history of PL.

It is now well established that cells bearing surface markers typical of B lymphocytes account for most of the increase in peripheral blood lymphocytes (PBL) in BLV-infected cows with PL. Muscoplat reported that unstimulated lymphocytes from BLV-infected cattle with PL incorporate large amounts of DNA precursor after 3 days in culture. Kenyon and Piper showed that the lymphocytes that replicate infectious BLV and those that spontaneously incorporate $3^H$-thymidine are distinct subpopulations of B lymphocytes. This observation, along with the finding that the B lymphocytes that spontaneously incorporate thymidine also synthesize $\mu$ chains, led Kenyon to suggest that these cells may represent a population of sensitized B cells committed in vivo to antibody production.
Glucocorticoids have an important position in the treatment of most lymphoid malignancies in man and animals. Little is known about the steroidal effects on lymphocyte populations in virus-induced lymphoproliferative disorders. In this study we examined the effects of glucocorticoids on PBL from both BLV-associated lymphoproliferative states. Changes following therapy were monitored in tissue culture as well as in vivo where the total numbers and subpopulations of PBL were measured. In doing so, we asked the following questions: (1) How do glucocorticoid effects on lymphocyte populations in BLV-infected cows with PL compare with those observed in cows with lymphosarcoma cell leukemia (BLSL)? (2) Can in vitro steroidal effects be correlated with those observed in vivo? (3) Does glucocorticoid therapy affect tumor progression in cows with BLSL?

MATERIALS AND METHODS

Animals

BLV-infected cows with PL were selected from Jersey herd BF in which approximately 60% of the animals have PL and 93% have detectable antibodies against the BLV internal antigen. Animals selected from this herd were clinically normal adult cows 7–11 yr of age with total lymphocyte counts ranging from 19,000 to 40,000 lymphocytes/cu mm, of which 83%–92% carried surface immunoglobulin (S-Ig) detectable by membrane immunofluorescence.

BLV-infected cows with lymphosarcoma were selected both from among the cows referred to New Bolton Center by veterinarians for study and from our own BF herd. In addition to solid tumor (lymphadenopathy and/or internal organ involvement), the cows selected had BLSL with morphologically atypical lymphocytes in the peripheral blood greater than 40,000/cu mm and were mature dairy cows 4–9 yr of age that were clinically stable for at least 3 mo prior to the initiation of in vivo studies.

All BLV-infected animals used in this study had antibodies to internal virus antigen, and their PBL induced syncytia in bovine embryo spleen monolayer cell cultures. Both groups of cows were carefully monitored at New Bolton Center for a period of 30 days prior to in vivo studies, during which time baseline data were collected.

Preparation of Lymphocytes

PBL were prepared using a modified method of Boyum. Ten-milliliter aliquots of heparinized blood collected aseptically by jugular venipuncture were diluted with 20 ml Earl’s balanced salt solution (EBSS) and carefully layered on 15 ml of sodium metrizoate (Hypaque)/Ficoll (Lymphoprep) (Nygaard, Oslo) in 50-ml plastic centrifuge tubes. The gradients were centrifuged for 30–40 min at 400 g at room temperature. The resulting band of cells at the interface was carefully collected and washed three times in cold EBSS. Cell suspensions prepared in this way contained more than 95% mononuclear cells, with at least 95% viability as determined by trypan blue dye exclusion.

Preparation of EAC Rosettes

EAC-rosette formation was tested by a modification of the method described by Bianco et al. Ten milliliters of sheep red blood cells (SRBC) collected into an equal volume of Alsever’s solution and stored up to 2 wk at 4°C before use were washed three times in EBSS. A subagglutinating dose of rabbit antiserum sheep hemolysin (GIBCO, Grand Island, N.Y.) was added and incubated at 37°C for 30 min. The SRBC were then washed twice in EBSS, resuspended in EBSS containing fresh horse complement at a dilution of 1:40, and incubated at 37°C for 1 hr. After washing twice in EBSS, the SRBC were adjusted to a 2% suspension and stored at 4°C up to 48 hr before use. One-tenth to 0.2 ml of the 2% suspension of sensitized SRBC was added to an equal volume of PBL at a concentration of 10⁷ cells/ml of medium 199, incubated at 37°C for 30 min, centrifuged for 5 min at 60 g, and gently resuspended following the addition of 1–2 drops 1% brilliant cresyl blue stain. One drop of the cell suspension was placed on a glass slide, and a cover slip was placed over the wet preparation. The percentages of mononuclear cells with three or more SRBC attached were determined from 4–200 cell counts performed using a phase microscope. Less than 1% of bovine lymphocytes were found to bind unsensitized SRBC.
Membrane Immunofluorescence

The presence of immunoglobulin on the surface of PBL was detected using a direct membrane immunofluorescence test previously described. At least 4–200 cell counts were performed using a Zeiss fluorescence microscope to determine the percentage of positive cells.

Discontinuous Density Gradients

Discontinuous albumin density-gradient centrifugation of peripheral blood leukocytes was performed according to a method described by Kenyon and Piper.

Presence of Antibodies Against the Internal Antigen of BLV

Demonstration of antibodies to the internal antigen of BLV was accomplished by an indirect fluorescent antibody technique, as described by Ferrer et al.

Lymphocyte Cultures

PBL separated by the Ficoll-Hypaque gradient technique were distributed in triplicate 2-ml cultures containing 10^6 cells in medium 199 supplemented with 20% pooled normal bovine serum (BLV negative or positive for the presence of antibodies against the internal antigen for BLV) that was inactivated at 56°C for 30 min, penicillin (100 U/ml), and streptomycin (100 µg/ml). Hydrocortisone 21-sodium succinate (HSS) (Sigma Chemical, St. Louis, Mo.) was added to the medium in concentrations ranging from 10^{-5} to 10^{-6} M. The HSS solutions were prepared using medium 199 and filtered through a 0.22-µ Millipore filter before being added to the culture medium.

Cultures stimulated with phytohemagglutinin (PHA, M form) and pokeweed mitogen (PWM) (GIBCO) were carried out using concentrations of 2.5-5µl/ml and 5-10µl/ml for the respective mitogens. Concentrations of PHA and PWM giving optimal ^4C-thymidine incorporation as determined by preliminary dose-response curves were used.

The lymphocyte cultures were harvested after 72 hr of incubation at 37°C in a humidified 5% CO2 atmosphere. ^4C-thymidine labeling was carried out for the terminal 18 hr of incubation following the addition of 0.1 µCi of ^4C-thymidine, specific activity 58.9 Ci/mrnole (New England Nuclear, Boston, Mass.), to each of the culture tubes. After incubation, the cells were collected on 2.4-cm glass fiber filters (grade 934AH, Reeve Angel, Clifton, N.J.), washed with 10 ml of saline (0.9 g/dl), 5 ml of cold TCA (5 g/dl), and 5 ml of absolute ethyl alcohol, dried under an infrared lamp for 10 min, placed in 5 ml of liquid scintillant [4.5 g of 2,5-diphenyloxazole (PPO) (Packard Instrument, Downers Grove, Ill.) and 0.45 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene (POPOP) (Packard Instrument) per liter of toluene], and counted in a Packard Tri-Carb liquid scintillation counter at a 76% counting efficiency. Incorporation of ^4C-thymidine is reported as mean counts per minute of the triplicate cultures.

Discontinuous BSA density-gradient-fractionated cells were cultured according to a method previously described.

In Vivo Studies

Three BLV-infected cows with PL and 3 cows with BLSL were each administered 1 g of prednisolone acetate (Rugby Laboratories, Rockville Center, N.Y.) intramuscularly divided into three treatments per day for 21 days. After day 21 the dose was tapered over 4 days. Daily blood samples were collected from either the jugular or tail vein during the treatment period and at regular intervals following the end of treatment. Three-milliliter aliquots of heparinized plasma prepared from these samples were stored at -20°C for up to 3 mo before being assayed for total corticoids. Plasma corticoid levels were kindly measured by Dr. V. K. Ganjam using a competitive protein-binding assay described in detail by Murphy. Baseline data were collected for a period of 30 days before treatment. The clinical course of each animal was carefully monitored during and after the treatment period, with special attention to solid tumor load, including peripheral lymphadenopathy, abdominal masses palpable per rectum, and clinical manifestations of internal organ involvement.

RESULTS

The spontaneous ^4C-thymidine incorporation by PBL from all 5 cows with PL was markedly inhibited by concentrations of HSS as low as 10^{-7} M (Table 1).
Inhibition of thymidine incorporation by HSS also occurred using PBL from 2 cows with BLSL. Animal BF-194, however, demonstrated a relative resistance to the corticoid, incorporating over 11,000 cpm in the presence of $10^{-5}$M HSS.

To determine whether or not populations of steroid-sensitive cells were present in BF-194 along with resistant lymphocytes, PBL from BF-194 and a cow with PL (BF-178) were further fractionated using a BSA discontinuous density gradient, and the effect of HSS on $^3$H-thymidine incorporation by each of the fractions collected was examined. Figure 1 compares the inhibitory effect of $10^{-7}$M HSS on

### Table 1. Effect of HSS on Spontaneous Incorporation of $^{3}$C-Thymidine In Vitro by PBL From BLV-Infected Cows

<table>
<thead>
<tr>
<th>Animal</th>
<th>Molar Concentration of HSS</th>
<th>0</th>
<th>$10^{-7}$</th>
<th>$10^{-6}$</th>
<th>$10^{-5}$</th>
<th>$10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows with PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF-157</td>
<td>29,760*</td>
<td>5,902</td>
<td>5,358</td>
<td>3,436</td>
<td>1,544</td>
<td></td>
</tr>
<tr>
<td>BF-237</td>
<td>19,350</td>
<td>2,652</td>
<td>2,433</td>
<td>2,000</td>
<td>696</td>
<td></td>
</tr>
<tr>
<td>BF-178</td>
<td>15,994</td>
<td>1,620</td>
<td>1,185</td>
<td>801</td>
<td>544</td>
<td></td>
</tr>
<tr>
<td>BF-238</td>
<td>24,554</td>
<td>1,100</td>
<td>1,545</td>
<td>1,464</td>
<td>906</td>
<td></td>
</tr>
<tr>
<td>BF-250</td>
<td>27,079</td>
<td>3,272</td>
<td>2,387</td>
<td>2,048</td>
<td>631</td>
<td></td>
</tr>
<tr>
<td>Cows with BLSL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-4</td>
<td>20,560</td>
<td>1,973</td>
<td>1,056</td>
<td>849</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>BF-194</td>
<td>28,100</td>
<td>13,287</td>
<td>11,061</td>
<td>11,351</td>
<td>5,066</td>
<td></td>
</tr>
</tbody>
</table>

*Counts per minute incorporated by $10^6$ cells cultured in medium supplemented with 20% homologous BLV-negative serum.

Inhibition of thymidine incorporation by HSS also occurred using PBL from 2 cows with BLSL. Animal BF-194, however, demonstrated a relative resistance to the corticoid, incorporating over 11,000 cpm in the presence of $10^{-5}$M HSS.

To determine whether or not populations of steroid-sensitive cells were present in BF-194 along with resistant lymphocytes, PBL from BF-194 and a cow with PL (BF-178) were further fractionated using a BSA discontinuous density gradient, and the effect of HSS on $^3$H-thymidine incorporation by each of the fractions collected was examined. Figure 1 compares the inhibitory effect of $10^{-7}$M HSS on

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** Comparison of the distributions through a BSA discontinuous density gradient of cells that spontaneously incorporate $^3$C-thymidine with and without $10^{-7}$M HSS in vitro from PL cow BF-178 (A) and those from BLSL cow BF-194 (B).
thymidine incorporation by gradient-separated fractions of PBL from the leukemic animal with that in BF-178. Although incorporation was markedly inhibited in all fractions of PBL from BF-178, both steroid-sensitive fractions and fractions that incorporated large amounts of $^{3}$H-thymidine in the presence of HSS were present in cells from BF-194.

Evidence presented in Table 2 demonstrates that mitogen-stimulated thymidine incorporation in vitro is considerably more resistant to $10^{-7}$ and $10^{-5}$M HSS than is spontaneous incorporation. Although the total thymidine incorporation in cultures with PHA and HSS added is less than in cultures without the steroid, the difference between incorporation by PHA-stimulated cells and incorporation by cells cultured without mitogen under the same conditions is not as consistently or as markedly reduced by the steroid. PWM-stimulated thymidine incorporation also occurred with HSS, although the values were reduced by 37%-78%.

Prednisolone acetate was administered to 3 cows with PL and 3 cows with BLSL over a 3-wk period. The absolute lymphocyte counts following administration of glucocorticoid to cows with PL were reduced to 10%-20% of the initial values by the end of the 21 days of treatment in all 3 animals (Fig. 2A). Furthermore, the counts remained at this level for at least 40 days and as long as 60 days following cessation of treatment before they began to rise.

Absolute lymphocyte counts following administration of prednisolone acetate to cows with BLSL are shown in Fig. 2B. Two of the 3 animals administered the steroid showed rapid and profound decreases in lymphocyte counts similar to those seen in the animals with PL. In the third animal (BF-194) the lymphocyte count did not decrease; in fact, it increased over the 21 days of treatment. All 3 cows underwent rapid progression of disease within 5 days of cessation of treatment. An expansion of tumor load in these animals was reflected by rapid enlargement of peripheral lymph nodes and clinical manifestations of internal involvement (including posterior paresis resulting from impingement of tumor on the spinal cord, congestive heart failure resulting from right heart involvement, and abomasal and/or intestinal dysfunction following infiltration of the walls of these organs). These observations were confirmed on postmortem examination. In addition, tumor

Table 2. Effect of HSS on PHA- and PWM-Stimulated $^{14}$C-Thymidine Incorporation In Vitro by PBL From BLV-Infected Cows With PL

<table>
<thead>
<tr>
<th>HSS Concentration (M)</th>
<th>PHA (Total cpm)</th>
<th>PWM (Total cpm)</th>
<th>No Mitogen (Total cpm)</th>
<th>PHA-Stimulated* (cpm)</th>
<th>PWM-Stimulated* (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal BF-250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30,839</td>
<td>51,699</td>
<td>19,269</td>
<td>11,570</td>
<td>32,430</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>11,855</td>
<td>22,386</td>
<td>1,949</td>
<td>9,906</td>
<td>20,437</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>13,454</td>
<td>14,072</td>
<td>914</td>
<td>12,540</td>
<td>13,158</td>
</tr>
<tr>
<td>Animal BF-238</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>79,070</td>
<td>128,439</td>
<td>32,964</td>
<td>46,106</td>
<td>95,475</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>64,659</td>
<td>47,931</td>
<td>2,034</td>
<td>62,625</td>
<td>45,897</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>52,579</td>
<td>22,889</td>
<td>1,505</td>
<td>51,074</td>
<td>21,384</td>
</tr>
<tr>
<td>Animal BF-237</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>63,798</td>
<td>67,365</td>
<td>9,457</td>
<td>54,341</td>
<td>57,908</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>42,510</td>
<td>34,285</td>
<td>552</td>
<td>41,858</td>
<td>33,733</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>35,006</td>
<td>25,201</td>
<td>436</td>
<td>34,570</td>
<td>24,765</td>
</tr>
</tbody>
</table>

*Total cpm minus cpm measured in cultures with no mitogen added.
expansion was associated with a rapid increase in the number of atypical lymphocytes in the peripheral blood. The circulating lymphoid cells at this time appeared more uniformly large, with loose chromatin and prominent multiple nucleoli (Fig. 3). Binucleated cells, mitotic figures, and cells with cleft nuclei were increased in numbers.

The numbers of circulating lymphocytes bearing B-cell membrane markers (complement receptors and/or surface immunoglobulin) were examined in BLV-infected cows both before and after glucocorticoid administration (Fig. 4). In the cows with PL the decrease in SEAC-rosette forming cells (RFC) and S-Ig-positive cells accounted for most (85%-98%) of the decrease in the total lymphocyte count. This was in contrast to the findings in the 2 BLSL cows, in which the decrease in B lymphocytes accounted for a relatively small proportion of the decrease in the total lymphocyte count.

Evidence that the circulating B-cell population reduced by the steroid is the same population of cells that spontaneously incorporates thymidine is presented in Fig. 5. Following 1 wk of treatment, spontaneous incorporation of 14C-thymidine by PBL
in vitro was reduced to 10%–20% of the pretreatment level in each of the 3 cows with PL. Lymphocytes from BLSL cow BF-194 continued to incorporate large amounts of thymidine following the same period of time. Prior to the end of the treatment period, thymidine incorporation by lymphocytes from both BLSL cows began to increase sharply. Following cessation of treatment, incorporation by cells from the leukemic animals rose to levels well beyond the pretreatment values. This increase corresponded with the influx of atypical cells into the circulation and solid
Fig. 4. Comparison of the decrease in numbers of circulating lymphocytes with B-cell markers following administration of prednisolone acetate in cows with PL and cows with BLSL.

Fig. 5. Spontaneous incorporation of $^{3}H$-thymidine in vitro by PBL from 3 cows with PL (A) and 2 cows with BLSL (B) before, during, and after the period of treatment with prednisolone acetate in vivo.
Table 3. Effect of In Vivo Administration of Prednisolone Acetate to Cows With BLSL on the Resistance of Spontaneous Incorporation of ¹⁴C-Thymidine by PBL to HSS In Vitro

<table>
<thead>
<tr>
<th>Animal</th>
<th>Day</th>
<th>Molar Concentration of HSS</th>
<th>0</th>
<th>10⁻¹</th>
<th>10⁻²</th>
<th>10⁻³</th>
<th>10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-4</td>
<td>Pretreatment</td>
<td>20.550*</td>
<td>1.973</td>
<td>1.056</td>
<td>849</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>57.222</td>
<td>53.582</td>
<td>55.508</td>
<td>56.000</td>
<td>37.232</td>
<td></td>
</tr>
<tr>
<td>BF-194</td>
<td>Pretreatment</td>
<td>28.100</td>
<td>13.287</td>
<td>11.061</td>
<td>11.351</td>
<td>5.066</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>76.086</td>
<td>53.977</td>
<td>46.274</td>
<td>45.519</td>
<td>17.366</td>
<td></td>
</tr>
</tbody>
</table>

*Counts per minute incorporated by 10⁶ cells cultured in medium supplemented with 20% homologous BLV-negative serum.

tumor expansion. Increases in thymidine incorporation over pretreatment levels were not observed in the 3 cows with PL. In fact, incorporation by PBL from 2 of the PL cows treated remained very low for as long as 1 mo following the end of the treatment period. Table 3 compares the inhibition of thymidine incorporation by HSS before treatment with that observed by the end of the treatment. Pretreatment studies show marked inhibition of spontaneous incorporation in vitro by PBL from cow S-4 at an HSS concentration of 10⁻⁷ M. Following 20 days of treatment, inhibition was less marked. PBL collected 10 days following cessation of treatment (day 35) were resistant to the steroid, incorporating large amounts of ¹⁴C-thymidine in the presence of concentrations of HSS as high as 10⁻⁴ M. BF-194, whose PBL had contained populations of both HSS-sensitive and HSS-resistant cells, as shown in Fig. 1, became increasingly resistant to the inhibitory effect of the steroid on thymidine incorporation in vitro when glucocorticoid treatment was discontinued. Following treatment, PBL from BF-194 were again spun through a discontinuous BSA density gradient, and the fractions collected were examined for their ability to incorporate thymidine in tissue culture with and without HSS (Fig. 6). In contrast to the pretreatment observations (Fig. 1), HSS-sensitive populations of cells were no longer present in the peripheral blood.

Evidence that a change in the density distribution of the circulating lymphocytes corresponded with the changes observed in morphology and resistance to HSS in vitro is presented in Fig. 7. The density distribution of cells from PL cow BF-178, when examined prior to administration of prednisolone acetate, showed a peak at fractions 3 and 4, with a smaller peak at fractions 8 and 9 comprised largely of...
granulocytes. Following 12 days of treatment, when the lymphocyte count had been reduced, the density distribution was examined and found again to peak at fractions 3 and 4, with a larger peak at fraction 8 caused by steroid-induced neutrophilia. The density distribution of leukocytes from BLSL cow BF-194 before treatment showed a peak at fractions 4 and 5. When the test was repeated, however, after glucocorticoids had been discontinued and the lymphocyte count had rapidly risen, there was a major peak at fractions 1 and 2, with a minor peak at fractions 3 and 4, representing a major shift in density to a less dense population of circulating lymphocytes.

**DISCUSSION**

The results of these experiments demonstrate that a large population of glucocorticoid-sensitive lymphocytes is present in the peripheral blood of BLV-infected cows with PL. Two lines of evidence support the contention that this lymphocyte population is identical to the expanded B-cell population that has recently been characterized: (1) Cows with PL treated with prednisolone acetate showed a marked reduction in their circulating lymphocytes that was due almost entirely to a decrease in lymphocytes with B-cell markers. (2) The ability of PBL from these animals to spontaneously incorporate large amounts of $^{14}$C-thymidine in vitro disappeared as the lymphocyte counts dropped, thus suggesting that the B cells affected by the steroid and the cells that spontaneously proliferated in tissue culture were the same lymphocyte subpopulation.

Previous observations suggest that normal bovine lymphocytes, in contradistinction to the expanded B-cell population in cows with PL, are relatively steroid-resistant. Consistent with these observations is the fact that the steroid-treated animals did not become lymphopenic; rather, their lymphocyte counts were reduced to within the normal range for an adult cow with a normal proportion of B cells. Furthermore, the response of PBL from cows with PL to PHA, a T-cell mitogen,
was relatively unaffected, thus suggesting that the PHA-responsive lymphocyte subpopulation was distinct from the steroid-sensitive B lymphocytes. Although PWM-stimulated thymidine incorporation was reduced in the presence of HSS, 22%-63% of the PWM-stimulated incorporation persisted, thus suggesting that perhaps both normal and steroid-sensitive reactive B lymphocytes may have been responding to this mitogen.

In vitro glucocorticoid effects on PBL from both cows with PL and cows with BLSL correlated well with those observed in vivo. The marked inhibitory effect of low concentrations of HSS on spontaneous incorporation by PBL from cows with PL correlated well with the in vivo effect of the corticoid, where a 90% decrease in total lymphocyte counts occurred even though elevated plasma corticoid levels were not maintained throughout the treatment period (Fig. 2). Similarly, BLSL cow S-4, whose PBL demonstrated steroid sensitivity prior to treatment, responded to the prednisolone acetate with a marked drop in the number of circulating lymphocytes and a reduction in solid tumor load by about 50%. PBL from BLSL cow BF-194 demonstrated a relative steroid resistance in vitro prior to treatment and responded to the drug neither clinically nor in terms of a reduction in the number of circulating tumor cells.

Following cessation of therapy, all 3 cows with BLSL underwent rapid disease progression and tumor expansion. Two of the 3 cows prior to the period of tumor progression experienced what appeared to be a period of tumor regression, with reductions in size of peripheral lymph nodes and abdominal tumor masses palpable rectally and a decrease in the number of circulating lymphocytes. BF-194 did not respond in this way to treatment. Yet, although the total lymphocyte count of BF-194 did not decrease, three lines of evidence suggest that major changes in the composition of the circulating lymphocytes took place: (1) Circulating lymphoid cells appeared more uniformly atypical following the treatment period, as compared with the lymphocytes in the blood prior to therapy. (2) The cells present in the circulation following cessation of treatment were more steroid-resistant, as reflected by the ability to incorporate large amounts of ¹⁴C-thymidine in the presence of high levels of HSS in vitro. (3) The circulating lymphoid cells following treatment were considerably less dense than the PBL prior to glucocorticoid administration. None of these changes was observed in PL animals treated with corticoid. These results suggest that steroid-sensitive lymphocytes are present in cows with BLSL and that a reduction in these cells is associated with rapid tumor progression.

Steroid-sensitive populations of lymphocytes in cows with BLSL may include the same subpopulation of cells that comprise the majority of circulating lymphocytes in BLV-infected cows with PL. This lymphocyte population may represent an important host defense mechanism against viral antigens or transformed lymphocytes that is removed during the course of steroid therapy, thus permitting rapid tumor growth. Several observations support these hypotheses. B lymphocytes that spontaneously proliferate in tissue culture are selectively depleted from the circulation of cows with PL administered glucocorticoids. Morphologically similar cells of comparable density are depleted from the blood of cows with BLSL treated similarly. Although most of the decrease in PBL following treatment was due to a decrease in non-B cells in the 2 BLSL animals with non-B-cell leukemia (suggest-
ing that the tumor cells were initially steroid-sensitive), there was also a decrease of 5000–8500 in the number of circulating B lymphocytes (Fig. 7). Steroid-sensitive as well as steroid-resistant lymphocytes that spontaneously incorporate \(^{14}\)C-thymidine in vitro may be present in the circulation of cows with BLSL. The dramatic increase in the rate of tumor growth following removal of the steroid-sensitive cells cannot be explained merely by a conversion from a steroid-sensitive to a steroid-resistant tumor unless the corticoid also exerts some permissive effect on tumor growth. Finally, many animals with PL that develop lymphosarcoma experience a dramatic fall in absolute lymphocyte count at the time of tumor development.\(^{6,26}\)

There is evidence that the fall is due primarily to loss of the spontaneously incorporating B-cell population while the BLV-infected population is unaffected.\(^6\)

The expanded population of B lymphocytes that spontaneously incorporates thymidine in vitro may be a population of sensitized cells committed in vivo to the production of antibodies against BLV. The observation by Ferrer et al.\(^{15}\) that cattle with PL have higher titers of antibody against BLV than virus-infected cattle without PL supports this hypothesis. In addition, Kenyon has observed that a substantial number of the PBL in cows with PL produce \(\mu\) chains and that this population is comparable in size to the population that spontaneously incorporates thymidine.\(^7\) The fact that the blastogenic activity of these cells is inhibited by serum from BLV-infected animals\(^7\) further suggests that these cells express BLV-related surface moieties and probably represents an immunologic response to viral antigens or tumor cells.

The major cellular response to BLV infection in the cow is that of the B lymphocyte. Although the immunologic specificity of the heavy chains produced by the reactive B lymphocytes found in BLV-infected cows has not yet been determined, the regulation of these cells by a serum factor present only in BLV-infected animals suggests that this response is immunologically specific. To hypothesize that an expanded subpopulation of B lymphocytes is playing a major role in the containment of tumor cells is contrary to the view that this is a role generally reserved for killer T cells. Yet several observations suggest that such a B-cell-mediated response to a lymphoid malignancy can occur. Such a response might indeed be antibody-dependent lymphocyte cytotoxicity.\(^{27,30}\) The question whether or not the steroid-sensitive B-lymphocyte population found in BLV-infected cows has this specific function awaits further investigation.

Our findings, along with those of other recent studies,\(^2,7,29\) have served to further define lymphocyte populations in BLV-infected cattle. It would be of interest to apply this information to studies of glucocorticoid action as well as studies of the immunology of BLV infection and its related malignancy. As a source of a relatively homogeneous population of glucocorticoid-sensitive lymphocytes, cows with PL may prove valuable in studies of the mechanism of action of corticoids on lymphoid cells. In addition, the apparent specificity of this action in the cow for a reactive cell population associated with BLV infection may provide a useful tool for examining the role these cells play in the immune response to the oncogenic virus. The fact that glucocorticoid therapy in cows with BLSL is associated with an acceleration of tumor growth further suggests that these drugs may be helpful in studying mechanisms of tumor containment in animals with this virus-associated malignancy.
REFERENCES


21. Murphy BET: Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J Clin Endocrinol Metab 27:973, 1969


26. Abt DA: Personal communication


28. Longmire RL, Ryan S, McMillan R: Anti-


Glucocorticoid effects on peripheral blood lymphocytes in cows infected with bovine leukemia virus

JC Bloom, SJ Kenyon and TG Gabuzda