Defective function of leukocytes from cattle persistently infected with bovine viral diarrhea virus, and the influence of recombinant cytokines

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Abstract
Cattle persistently infected with bovine viral diarrhea (BVD) virus have decreased neutrophil and lymphocyte functions. We reevaluated these functions and further characterized the inhibition of persistent BVD virus infection in neutrophils, using sensitive kinetic assays. In addition, the influence of in vitro incubation of neutrophils with recombinant bovine interferon gamma (~IloIFN gamma) and in vitro incubation of lymphocytes with recombinant bovine interleukin-2 was evaluated.

Disciplines
Comparative and Laboratory Animal Medicine | Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology

Comments

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Defective function of leukocytes from cattle persistently infected with bovine viral diarrhea virus, and the influence of recombinant cytokines

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SUMMARY

Cattle persistently infected with bovine viral diarrhea (BVD) virus have decreased neutrophil and lymphocyte functions. We reevaluated these functions and further characterized the inhibition of persistent BVD virus infection in neutrophils, using sensitive kinetic assays. In addition, the influence of in vitro incubation of neutrophils with recombinant bovine interleukin-2 was evaluated.

Significant (P < 0.05) decrease in random migration under agarose, Staphylococcus aureus ingestion, cytochrome-C reduction, iodination, antibody-independent cell-mediated cytotoxicity, oxidant production, and cytoplasmic calcium flux were observed in neutrophils from cattle persistently infected with BVD virus, compared with noninfected control cattle. Incubation of neutrophils from noninfected controls with rBoIFN gamma significantly (P < 0.05) decreased random migration under agarose, cytochrome-C reduction, and cytoplasmic calcium flux. Neutrophils from cattle persistently infected with BVD virus also had decreased random migration under agarose after incubation with rBoIFN gamma; in addition, antibody-independent cell-mediated cytotoxicity, elastase release, and cytoplasmic calcium flux were significantly enhanced. The rBoIFN gamma induced significantly (P < 0.05) different effects on chemotaxis, cytochrome-C reduction, iodination, and cytoplasmic calcium flux of neutrophils from infected and control cattle. The rBoIFN gamma was more effective at improving the function of neutrophils from cattle persistently infected with BVD virus, compared with neutrophils from controls.

Lymphocytes from infected cattle had decreased blastogenesis in response to phytohemagglutinin, concanavalin A, and pokeweed mitogen. Incubation of those lymphocytes with recombinant bovine interleukin-2, with no mitogen present, significantly (P < 0.05) increased incorporation of [3H]thymidine. However, the response of lymphocytes to mitogen stimulation was not significantly increased by the presence of recombinant bovine interleukin-2, indicating that depression of in vitro lymphocyte blastogenesis in the cattle persistently infected with BVD virus is not attributable to decreased production of interleukin-2.

Bovine viral diarrhea (BVD) virus is classified as a pestivirus in the Togaviridae family. The virus is ubiquitous in the cattle population of North America and is virulent in its cytopathic and noncytopathic forms. Bovine viral diarrhea has many clinical manifestations including: mild or subclinical infection (the most common form), persistent infection, mucosal disease, and chronic BVD.1-3

Persistent BVD virus infection develops when a noncytopathic virus infects the fetus before 125 days of gestation (before the fetus is immunocompetent). The fetus becomes immunotolerant to the virus and does not produce antibodies to it.4 At birth, persistently infected calves have constant viremia and serve as natural reservoirs of the virus.4,6 Clinical signs of persistent BVD virus infection include decreased weight gain and stunted growth; however, many persistently infected calves grow normally and do not have clinical signs of infection.4,6

Mucosal disease is induced when a bovid persistently infected with noncytopathic BVD virus is co-infected with an appropriate cytopathic BVD virus.7,8 Mucosal disease is characterized by gastrointestinal abnormalities (ie, oral lesions and profuse diarrhea). The virus has an affinity for the cells of lymphoid tissue, and often lymphopenia and neutropenia are observed. It is not known whether the lymphopenia and neutropenia are attributable to viral infection of the bone marrow, to soluble factors that have an effect on the bone marrow, or to destruction of lymphocytes and neutrophils. Death from mucosal disease usually occurs within 3 to 10 days of onset of clinical signs of the disease.1 Chronic BVD is also a disease of high mortality. However, cattle with chronic BVD are severely emaciated and lame and have intermittent or constant diarrhea. Cattle with chronic BVD may survive up to 18 months.1,3

The BVD virus is immunosuppressive in cattle, affecting the function of several cell types. Similar to findings associated with other viral infections, cattle infected with BVD virus have decreased circulating lymphocyte and neutrophil numbers.9-11 However, immunosuppression is...
not entirely a result of decreased numbers. The immuno-suppression observed in cattle with BVD virus infection includes decreased mitogen-induced lymphocyte blastogenesis,1213 decreased monocyte chemotaxis,12 decreased ingestion of Staphylococcus aureus by neutrophils,14 decreased iodination (myeloperoxidase-H2O2-halide activity) in neutrophils,1115 and decreased antibody-dependent cell-mediated cytoxicity (ADCC) by neutrophils.16 The basic mechanism of this viral-induced immunosuppression is not clear, but it predisposes infected cattle to secondary bacterial infections or other viral infections.161718

We specifically were interested in investigating the immunosuppression observed in cattle with persistent BVD virus infection. It has been shown that persistently infected cattle have decreased lymphocyte blastogenesis and decreased S aureus ingestion by neutrophils.18 The primary objective of the study reported here was to better characterize the suppression of neutrophil function in cattle with persistent BVD virus infection. Using kinetic assays for studying neutrophil function, we were able to better define the suppressive effects of persistent BVD virus infection on neutrophils. Our second objective was to determine the effect of recombinant bovine interferon gamma (rBoIFN gamma) on in vitro function of neutrophils and the effect of recombinant bovine interleukin-2 (rBo-T2) on blastogenesis of blood lymphocytes obtained from cattle persistently infected with the virus.

Materials and Methods

Cattle—Nine healthy 1- to 2-year-old Holstein steers housed at Iowa State University served as controls. Nine cattle of mixed breeds persistently infected with BVD virus (age, 1 to 6 years, 6 females and 3 steers; 3 of the females were in midgestation) were part of the herd at the National Animal Disease Center, Ames, Iowa. Four of the cattle were persistently infected with virus isolate TGAN, and 4 of the cattle were persistently infected with virus isolate NEB. These cattle were experimentally infected during gestation. The other bovid was infected with Tiran, and 4 of the cattle were persistently infected with BVD virus isolate 9789, and was detected as being naturally infected and the control cattle, using an electronic cell counter. Values were determined on each of 5 days.

Kinetic assays of neutrophil function—Kinetic assays measuring oxidant production, elastase release, and cytoplasmic calcium flux were performed, using a photon-counting spectrophotometer.19 The neutrophil stimulant used in all assays was zymosan A 0 opsonized with fresh bovine serum, as described.20 Opsonized zymosan was used at a final concentration of 1.0 mg/ml.

Oxidant production—This assay, a modification of the procedure described by Hyslop and Sklar21 indirectly measures O2– production by neutrophils, as described.22 When neutrophils are appropriately stimulated and O2– is produced, it is rapidly converted to H2O2 in the presence of superoxide dismutase. In the presence of horseradish peroxidase, H2O2 oxidizes p-hydroxyphenylacetate (HPHA) to a fluorescent product PHPA, which emits light at a 400-nm wavelength when excited by light at a 340-nm wavelength. Each cuvette contained 2.5 ml of Hank’s balanced salt solution (PBSS) without phenol red, 5 x 106 neutrophils, and 75 µl of a reagent cocktail consisting of superoxide dismutase 2 (8 mg/ml of PBSS), horseradish peroxidase 2 (8 mg/ml of PBSS), and PHPA 2 (10 mg/ml of PBSS) at a ratio of 10:10:25, respectively. The assay was performed, using a program that allowed testing of 10 samples simultaneously, taking fluorescence readings of each sample every 150 seconds for 600 seconds. Readings included a baseline reading, then a time-zero reading, which measured the fluorescence just after the stimulant was added.

Elastase release assay—Elastase is an enzyme contained in neutrophil granules. The procedure described by Sklar et al23 for measuring elastase release was used as modified.24 The enzyme acts on the substrate methylenebis(cyanalanylalanylprolylvalylaminomethyl-coumarin (MCA), which when cleaved, liberates a fluorescent product anion methylcoumarin. Aminomethylcoumarin, when excited by light at a 400-nm wavelength, fluoresces at a 490-nm wavelength. Each cuvette for this assay contained 2.5 ml of Hank’s balanced salt solution (PBSS) at a light of 100 ng/ml. 5 x 106 neutrophils, and 75 µl of a reagent cocktail consisting of superoxide dismutase 2 (8 mg/ml of PBSS), horseradish peroxidase 2 (8 mg/ml of PBSS), and PHPA 2 (10 mg/ml of PBSS) at a ratio of 10:10:25, respectively. The MCA had been dissolved in dimethylsulfoxide, then diluted in PBSS to stock concentration of 1.25 mg/ml and was stored at –20 C. The assay was performed, using a similar program as described for the oxidant assay.

Cytoplasmic calcium fluxes—Fura 2/AM [1-[3-(6-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-[2-amino-5’-methyl-phenoxy]-ethane-N,N,N’-tetraacetic acid, pentacetoxy-methyl ester] is a fluorescent calcium indicator used to determine cytoplasmic calcium fluxes, as described.25 Neutrophils were incubated with Fura 2-AM at a concentration of 106 cells/ml. Five hundred microliters of the cell suspension was incubated for 2 hours with an equal volume of medium 199, with or without rBoIFN gamma. The final concentration of rBoIFN gamma in the neutrophil solution was 5.0 x 10–9 g/ml.

Total WBC count was determined for the persistently infected and the control cattle, using an electronic cell counter. Values were determined on each of 5 days.
for 30 minutes. During incubation, Fura 2-AM enters the cell and is hydrolysed by intracellular enzymes, thus trapping Fura 2 inside the cells. The peak absorbance of Fura 2 shifts from 380-nm to 340-nm wavelength after binding calcium. Therefore, the ratio of free to bound calcium can be measured during activation of neutrophils by opsonized zymosan. The aforementioned spectrofluorometer alternates the excitation wavelength between 340 nm and 360 nm every 2 seconds and records the emittance at a 510-nm wavelength. The corrected ratio of fluorescence (340 nm:380 nm) is stored by the computer. Each cuvette contained 2.5 ml of HSS and 5 x 10^6 Fura 2-loaded neutrophils. The cuvette was placed into the sample chamber, and the cells were stimulated 10 seconds after initiation of the assay. Fluorescence ratio was recorded every 2 seconds for 100 seconds. Calcium concentration can be determined from the fluorescence ratio as described.

Other neutrophil function assays—Additional assays were performed as described to evaluate neutrophil function in cattle persistently infected with BVD virus and the effect of in vitro incubation with rBovIFN gamma. Briefly, random migration under agarose was measured after an incubation period of 18 hours; the area of random migration was reported in square millimeters. 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 iododeoxyuridine S aureus. Neutrophils were incubated for 10 minutes with bacterial 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 30 minutes' incubation of neutrophils with cytochrome-C and opsonized zymosan. Results were reported as optical density/1.25 x 10^6 neutrophils/30 min. The iodination reaction, a measure of the myeloperoxidase-H_2O_2-halide system, was measured by incubating neutrophils with opsonized zymosan and NaI for 20 minutes; the reaction was terminated by addition of trichloroacetic acid. Results were reported as nanomoles of NaI/10^7 neutrophils/h. Antibody-dependent cell-mediated cytotoxicity was evaluated, using antibody-coated ⁵¹Cr-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-hour incubation. Antibody-independent cell-mediated cytotoxicity (ADCC) was measured similarly to ADCC; however, antibody was not added.

Lymphocyte blastogenesis evaluation—Lymphocytes were isolated from blood, and lymphocyte blastogenesis was performed, using pokeweed mitogen (PWM), phytohemagglutinin (PHA), and concanavalin A (conA) as mitogens, with a 72-hour incubation period as described. Mitogens were used at concentrations that resulted in optimal stimulation of lymphocytes from control cattle (final dilution of mitogen as supplied by the manufacturer: PHA, 1:1,000; conA, 1:2,000; PWM, 1:100). In addition, rBoIFN-2 was evaluated in vitro for its effect on blastogenesis of lymphocytes from cattle persistently infected with BVD virus and from controls. Lymphocytes were incubated with or without 10.0 ng of rBoIFN-2/ml.

Statistical analysis—Data were analyzed by use of a computerized program. Analysis of variance was performed, using a split-plot experimental design, with BVD virus infection status as the whole plot and lymphokine (rBovIFN gamma or rBoIFN-2) treatment as the subplot. Date of the assay was used as a blocking factor for all assays and, in addition, time point of data collection was used as a blocking factor for the kinetic neutrophil assays. The main effects of BVD virus infection status and lymphokine treatment were evaluated. Because a significant interaction was detected in a number of instances, the direct effect of lymphokine treatment on cells from either control or BVD virus-infected cattle was also evaluated, using one-way analysis of variance with split-plot design. A value of P < 0.05 was used to determine significance.

Results

Total WBC count The WBC count in persistently infected cattle was consistently lower than values in controls. In cattle of the control group, values ranged from 4,400 cells/µl to 14,000 cells/µl, with mean of 8,700 cells/µl and average differential WBC count of 41% neutrophils, 58% lymphocytes, and 2% eosinophils. Values in cattle persistently infected with BVD virus ranged from 2,520 cells/µl to 9,900 cells/µl, with mean of 4,300 cells/µl and average differential WBC count of 22% neutrophils, 78% lymphocytes, and 2% eosinophils.

Neutrophil assays—Results of assays examining the effects of persistent BVD virus infection, rBovIFN gamma, and the interaction of these 2 factors on neutrophil functions were determined (Tables 1 and 2; Fig 1–3). When compar-

<table>
<thead>
<tr>
<th>Table 1—Bovine neutrophil assay mean values for four treatment groups and the SEM for each assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil function</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Treatment group assay values</td>
</tr>
<tr>
<td>Random migration (mm²)</td>
</tr>
<tr>
<td>Chemotaxis ratio</td>
</tr>
<tr>
<td>Staphylococcus aureus ingestion (%)</td>
</tr>
<tr>
<td>Cytochrome-C reduction (OD)</td>
</tr>
<tr>
<td>Iodination (nmol of NaI/107 neutrophils/h)</td>
</tr>
<tr>
<td>Antibody-independent cell-mediated cytotoxicity (%)</td>
</tr>
</tbody>
</table>

rBoIFN gamma = recombinant bovine interferon gamma; PBVD = persistent bovine viral diarrhea virus infection. OD = optical density.
Table 2—Level of significance for the main effects of rBoIFN gamma and rBoIL-2 gamma on neutrophils and their interaction, using analysis of variance

<table>
<thead>
<tr>
<th>Neutrophil function</th>
<th>Control vs PBVD</th>
<th>PBVD vs rBoIFN gamma</th>
<th>rBoIFN gamma by PBVD interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random migration</td>
<td>0.048</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>0.71</td>
<td>0.13</td>
<td>0.68</td>
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<tr>
<td>S. aureus ingestion</td>
<td>0.001</td>
<td>0.44</td>
<td>0.26</td>
</tr>
<tr>
<td>Cytochrome-C reduction</td>
<td>0.001</td>
<td>0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>Iodination</td>
<td>0.03</td>
<td>0.52</td>
<td>0.37</td>
</tr>
<tr>
<td>Antibody-independent cell-mediated cytotoxicity</td>
<td>0.002</td>
<td>0.69</td>
<td>0.02</td>
</tr>
<tr>
<td>Antibody-dependent cell-mediated cytotoxicity</td>
<td>0.32</td>
<td>0.33</td>
<td>0.17</td>
</tr>
<tr>
<td>Oxidant production</td>
<td>0.03</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td>Elastase release</td>
<td>0.06</td>
<td>0.14</td>
<td>0.002</td>
</tr>
<tr>
<td>Cytoplasmic Ca²⁺ increase</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*See Table 1 for key.*

Figure 1—Mean values (n = 18) for the kinetic assay measuring oxidant production by neutrophils from 4 treatment groups. Mean values are the change in fluorescence units from baseline at time 0. The SEM for these mean values is 630 relative fluorescent units. PBVD = persistent bovine viral diarrhea virus = (recombinant bovine interferon gamma).

Figure 2—Mean values (n = 18) for the kinetic assay measuring elastase release from bovine neutrophils for 4 treatment groups. The mean values are the change in fluorescence units from baseline at time 0. The SEM for these mean values is 400 relative fluorescence units. See Figure 1 for key.

Figure 3—Mean values for the kinetic assay measuring cytoplasmic calcium fluxes in neutrophils from 4 treatment groups. Mean values represent the corrected fluorescence ratio (excitation, 340 nm/380 nm); SEM is 0.13. See Figure 1 for key.

pared with findings in controls, neutrophils from cattle persistently infected with BVD virus had significantly decreased random migration under agarose, ingestion of S. aureus, cytochrome-C reduction, iodination, AINC, oxidant production, and intracellular calcium flux. In addition, a tendency (P = 0.06) for decreased elastase release was apparent.

Neutrophils obtained from control cattle and incubated with rBoIFN gamma resulted in significantly decreased random migration under agarose, cytochrome-C reduction, and cytoplasmic calcium flux (Tables 1 and 2; Fig 3).

Neutrophils obtained from persistently infected cattle and incubated with rBoIFN gamma resulted in significant decrease in random migration under agarose, and increase of the following functions: AINC, elastase release, and cytoplasmic calcium flux (Tables 1 and 2; Fig 2 and 3).

The interaction between persistent BVD virus infection and rBoIFN gamma was statistically evaluated (Table 2). Significant interaction was observed between persistent infection and in vitro incubation with rBoIFN gamma for chemotaxis, cytochrome-C reduction, iodination, and cytoplasmic calcium flux. After incubation with rBoIFN gamma, neutrophils from cattle persistently infected with BVD virus had decreased chemotaxis and increased functions of cytochrome-C reduction, iodination and cytoplasmic calcium flux. Incubation with rBoIFN gamma had the opposite effect on neutrophils from control cattle for these assays.

Lymphocyte blastogenesis—Lymphocytes from persistently infected cattle had significantly decreased response to stimulation by PHA, conA, and PWM (Table 3). Incubation media that contained rBoIL-2 significantly (P < 0.01) increased the background counts for lymphocytes from control and persistently infected cattle, but did not increase mitogen-stimulated responses. Significant interaction between rBoIL-2 and persistent BVD virus infection was not detected.
Tritiated thymidine uptake by resting or stimulated blood lymphocytes from four treatment groups and level of statistical significance of the main effects of PBVD virus infection and recombinant bovine interleukin-2 (rBoIL-2) on lymphocytes and their interaction, using analysis of variance.

<table>
<thead>
<tr>
<th>Treatment group assay values</th>
<th>Probability of &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control vs PBVD</td>
</tr>
<tr>
<td></td>
<td>rBoIL-2</td>
</tr>
<tr>
<td>Mitogen</td>
<td>cpm</td>
</tr>
<tr>
<td>None</td>
<td>700</td>
</tr>
<tr>
<td>ConA</td>
<td>22,700</td>
</tr>
<tr>
<td>PWM</td>
<td>49,100</td>
</tr>
<tr>
<td>rBoIL-2</td>
<td>17,300</td>
</tr>
</tbody>
</table>

Results, the effect of rBoIFN gamma on neutrophils from healthy cattle is significantly different from the effect on neutrophils from cattle with persistent PBVD virus infection.

The mechanisms for the decreased cytoplasmic calcium flux and decrease in other neutrophil functions of cattle persistently infected with PBVD virus is unknown. Possibly, a decrease in cell surface receptors or an alteration in one of many steps in signal transduction is involved.

The signal transduction pathway that results after an opsonized particle binds to a bovine neutrophil has not been clearly defined. However, some possible explanations for the defects observed in neutrophils of persistently infected cattle can be made on the basis of what is known of the signal transduction pathway of human neutrophils. Briefly, an opsonized particle binds to a receptor that signals a G protein. The G protein activates an enzyme that cleaves membrane lipids, resulting in formation of a cytosolic messenger (inositol triphosphate) and a lipid soluble messenger (diacylglycerol). The cytosolic portion signals calcium to be released from intracellular stores, and the membrane portion stimulates protein kinase C. The importance of the cytosolic calcium flux has been reported for many neutrophil functions. The calcium flux is one of the initial events in receptor-mediated activation, and is essential for subsequent cellular responses. It is likely that the decreased cytoplasmic calcium flux in neutrophils from cattle persistently infected with PBVD virus contributes to other defective functions. This is somewhat supported by the concurrent improvement in cytoplasmic calcium flux and several other functions of neutrophils from persistently infected cattle after incubation with rBoIFN gamma. Recombinant BoIFN gamma may reverse the defective step(s) in the signal transduction pathway or may act at another step.

Compared with lymphocytes from control cattle, those from cattle persistently infected with PBVD virus had decreased lymphocyte blastogenesis in response to all 3 mitogens. These results were consistent with previous reports of decreased mitogen-induced blastogenesis in cattle with persistent PBVD virus infection. Other forms of PBVD virus infection also are reported to result in decreased lymphocyte blastogenic response to mitogens. The mechanism for the decrease in blastogenesis is not known.

Discussion

This investigation extended results of a previous study defining alterations of neutrophil and lymphocyte function in cattle persistently infected with PBVD virus. Additional aspects of neutrophil function were found to be suppressed, in vitro incubation with rBoIFN gamma was shown to significantly improve several of the suppressed functions. These results indicate that rBoIFN gamma may be effective in vivo for overcoming immunosuppression in cattle with persistent PBVD virus infection similarly as for cattle immunosuppressed by administration of dexamethasone. In vitro incubation with rBoIL-2 was not able to alter depression of lymphocyte blastogenesis in cattle persistently infected with PBVD virus, indicating that depression was not attributable to decreased production of IL-2 in vitro.

The influence of IFN gamma on neutrophils has been evaluated. Human neutrophils incubated with recombinant human IFN gamma have increased number of Fc receptors and enhanced phagocytic and cytotoxic functions. Interferon gamma (in vitro and in vivo) alters neutrophil functions of cattle, although results are variable. The reason for this variability is not clear. In general, rBoIFN gamma has a greater effect on the function of neutrophils obtained from cattle that are immunosuppressed, compared with neutrophils obtained from healthy control cattle. This is consistent with results of our study. Therefore, the variability observed in the effects of rBoIFN gamma on neutrophil function from clinically normal cattle may be attributable to variability in physiologic status of healthy cattle.

The mechanism of action of IFN gamma is not well described; however, it is known that IFN gamma binds to a cell surface receptor and increases production of ARN, elastase, and cytoplasmic calcium flux, and inhibited random migration. Incubation of rBoIFN gamma with neutrophils from control cattle also resulted in significantly inhibited random migration under agarose; however, cytochrome-C reduction and cytoplasmic calcium flux were decreased. Significant interaction between rBoIFN gamma and persistent PBVD virus infection was detected for neutrophil chemotaxis, cytochrome-C reduction, and cytoplasmic calcium increase. On the basis of our assay results, the effect of rBoIFN gamma on neutrophils from healthy cattle is significantly different from the effect on neutrophils from cattle with persistent PBVD virus infection.
function in these cattle persistently infected with BVD virus were similar to those described in a different group of cattle persistently infected with BVD virus indicates that the defects are associated with BVD virus infection.

Interleukin-2 is important for proliferation of T cells. Interleukin-2 binds to a receptor that activates protein kinase C, resulting in phosphorylation of proteins leading to DNA synthesis. One of the purposes of our study was to determine whether the decrease in lymphocyte blastogenesis of cattle persistently infected with BVD virus was attributable to decreased production and/or secretion of IL-2 from T cells, others reported that addition of IL-2 to lymphocyte cultures reverses the suppression of lymphocyte blastogenesis induced by cortisol. In our study, rBoIL-2 was mitogenic by itself, as indicated by its influence on proliferation (in the absence of mitogens) of lymphocytes from healthy cattle and cattle persistently infected with BVD. The fact that blastogenesis of lymphocytes from both groups of cattle was equally enhanced by rBoIL-2 (in the absence of mitogens) indicates that the lymphocyte defect may not be in the IL-2 stimulatory pathway. Enhancement of blastogenesis in nonstimulated lymphocytes by IL-2 has been described. An additive effect of rBoIL-2 and PHA or conA on lymphocytes from persistently infected cattle is not known; but is probably attributable to the same factors that limit lymphocyte proliferation in response to mitogens. The addition of rBoIL-2 to lymphocyte cultures did not significantly enhance the blastogenic response of these cells to mitogens, which indicates that a deficiency of IL-2 may not be responsible for the decreased blastogenic responsiveness of lymphocytes from infected cattle. The mechanism of suppression of lymphocyte blastogenesis is not known; however, because neutrophils and lymphocytes use a similar signal transduction pathway, it may be that a similar defect in signal transduction is responsible for inhibition of lymphocyte and neutrophil functions.

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