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

Gayle B. Brown, DVM, MS; Steve R. Bolin, DVM, PhD; Dagmar E. Frank, DVM, MS; James A. Roth, DVM, PhD

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 interferon gamma (rBoIFN gamma) and in vitro incubation of lymphocytes 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 [^{3}H]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.

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From the Department of Veterinary Microbiology and Preventive Medicine (Brown, Roth, Frank), Iowa State University, Ames, IA, 50011 and the National Animal Disease Center (Bolin), Midwest Area Agricultural Research Service, USDA, Ames, IA 50010.

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Address reprint requests to Dr. Roth.

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.[4,5] 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 immunosuppression observed in cattle with BVD virus infection includes decreased mitogen-induced lymphocyte blastogenesis,[12-16] decreased monocyte chemotaxis,[17] decreased ingestion of *Staphylococcus aureus* by neutrophils,[16] decreased iodination (myeloperoxidase-H_2O_2-halide activity) in neutrophils,[11,16] and decreased antibody-dependent cell-mediated cytotoxicity (ADCC) by neutrophils.[15] The basic mechanism of this viral-induced immunosuppression is not clear, but it predisposes infected cattle to secondary bacterial infections or other viral infections.[18-20]

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.[16] 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 (rBoIL-2) 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 virus isolate 9789, and was detected as being naturally persistently infected at 6 months of age; clinical evidence of infection was lacking.

Lymphokine preparations—The rBoIFN gamma[a] was supplied. The preparation contained 12.5 mg of protein/ml and had specific activity of approximately 2.2×10^6 U/ml. The rBoIL-2[b] was obtained elsewhere. The preparation had 1.99 μg of protein/ml and specific activity of approximately 20,000 U/ml.

Neutrophil isolation—Bovine neutrophils were isolated as described.[21] Briefly, 250 ml of blood was withdrawn from control cattle and 500 ml of blood was withdrawn from persistently infected cattle into bottles containing acid-citrate-dextrose anticoagulant. After centrifugation, the plasma was discarded and the buffy coat was removed and used to isolate lymphocytes. Packed RBC were lysed with cold buffered hypotonic solution for 1 minute, then isotonicity was restored. Cells were pelleted, and the lysing step was repeated. The isolated neutrophils were suspended in 1.5*M* phosphate-buffered saline solution (PBSS) at a concentration of 10^8 cells/ml. Five hundred microliters of the cell suspension was incubated for 2 hours with an equal volume of medium 199,[c] with or without rBoIFN gamma. The final concentration of rBoIFN gamma in the neutrophil solution was 5.0×10^{-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.

Kinetic assays of neutrophil function—Kinetic assays measuring oxidant production, elastase release, and cytoplasmic calcium flux were performed, using a photon-counting spectrofluorometer.[d] The neutrophil stimulant used in all assays was zymosan A[e] opsonized with fresh bovine serum, as described.[21] 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 Sklar[22] indirectly measures O_2^- production by neutrophils, as described.[23] When neutrophils are appropriately stimulated and O_2^- is produced, it is rapidly converted to H_2O_2 in the presence of superoxide dismutase. In the presence of horseradish peroxidase, H_2O_2 oxidizes p-hydroxyphenylacetate (PHPA) to a fluorescent product $PHPA_2$, which emits light at a 400-nm wavelength when excited by light at a 340-nm wavelength. Each cuvette contained 2.5 ml of Hanks balanced salt solution (HBSS) without phenol red,[c] 5×10^6 neutrophils, and 75 μl of a reagent cocktail consisting of superoxide dismutase[e] (8 mg/ml of PBSS), horseradish peroxidase[e] (8 mg/ml of PBSS), and PHPA[e] (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-0 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 al[24] for measuring elastase release was used as modified.[23] The enzyme acts on the substrate methylsuccinyl-alanylalanylprolylvalylaminomethyl-coumarin (MCA), which when cleaved, liberates a fluorescent product aminomethylcoumarin. Aminomethylcoumarin, when excited by light at a 380-nm wavelength, fluoresces at a 490-nm wavelength. Each cuvette for this assay contained 2.5 ml of HBSS, 5×10^6 neutrophils, and 75 μl of MCA.[f] 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-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methyl-phenoxy)-ethane-*N,N,N′N′*-tetraacetic acid, pentaacetoxymethyl ester} is a fluorescent calcium indicator used to determine cytoplasmic calcium fluxes, as described.[23] Neutrophils were incubated with Fura 2-AM

[a] Lot No. 322/38, Ciba-Geigy Ltd, Basel, Switzerland.
[b] Lot No. 21309910, Immunex Corp, Seattle, Wash.
[c] GIBCO, Grand Island, NY.
[d] Model 8000C, SLM Instruments Inc, Urbana, Ill.
[e] Sigma Chemical Co, St Louis, Mo.
[f] Peninsula Laboratories Inc, Belmont, Calif.

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 380 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 HBSS and 5×10^6 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.[25]

Other neutrophil function assays—Additional assays were performed as described[16,24] to evaluate neutrophil function in cattle persistently infected with BVD virus and the effect of in vitro incubation with rBoIFN 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 [^{125}I]iododeoxyuridine *S aureus*. Neutrophils were incubated for 10 minutes with bacteria at a ratio of 60:1 (bacteria to neutrophil), then lysostaphin was added to remove the extracellular *S aureus*; results were reported as percentage of bacteria ingested.

Reduction of cytochrome-C, a measure of superoxide anion production, was evaluated after 30 minutes' incubation of neutrophils with cytochrome-C and opsonized zymosan. Results were reported as optical density/1.25×10^6 neutrophils/30 min. The iodination reaction, a measure of the myleoperoxidase-H_2O_2-halide system, was measured by incubating neutrophils with opsonized zymosan and Na[^{125}I] 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 ^{51}Cr-labeled chicken RBC as the target cell. The effector-to-target cell ratio was 10:1, and results were reported as percentage of specific release during a 2-hour incubation. Antibody-independent cell-mediated cytotoxicity (AINC) 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),[c] phytohemagglutinin (PHA),[g] and concanavalin A (conA)[e] as mitogens, with a 72-hour incubation period as described.[15] 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, rBoIL-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 rBoIL-2[h]/ml.

Statistical analysis—Data were analyzed by use of a computerized program.[26] Analysis of variance was performed, using a split-plot experimental design, with BVD virus infection status as the whole plot and lymphokine (rBoIFN gamma or rBoIL-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, 76% lymphocytes, and 2% eosinophils.

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

[g] Difco Laboratories, Detroit, Mich.

[h] Immunex Corp, Seattle, Wash.

Table 1—Bovine neutrophil assay mean values for four treatment groups and the SEM for each assay

	Treatment group assay values				
Neutrophil function	Control (n = 18)	Control + rBoIFN gamma (n = 18)	PBVD (n = 18)	PBVD + rBoIFN gamma (n = 18)	SEM
Random migration (mm^2)	60	44	48	38	±3
Chemotaxis ratio	1.33	1.36	1.44	1.25	±0.04
Staphylococcus aureus ingestion (%)	29	31	21	24	±1
Cytochrome-C reduction (OD)	0.87	0.80	0.74	0.80	±0.02
Iodination (nmol of NaI/10^7 neutrophils/h)	34	31	25	27	±1
Antibody-independent cell-mediated cytotoxicity (%)	16	18	2	8	±1
Antibody-dependent cell-mediated cytotoxicity (%)	52	57	48	54	±3

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 PBVD virus infection and rBoIFN gamma on neutrophils and their interaction, using analysis of variance

Neutrophil function	Control vs PBVD	Control vs control + rBoIFN gamma	PBVD vs PBVD + rBoIFN gamma	PBVD by rBoIFN gamma interaction
Random migration	0.048	0.001	0.03	0.57
Chemotaxis	0.71	0.13	0.08	0.02
S aureus ingestion	0.001	0.44	0.25	0.99
Cytochrome-C reduction	0.001	0.01	0.13	0.001
Iodination	0.03	0.50	0.37	0.01
Antibody-independent cell-mediated cytotoxicity	0.002	0.69	0.02	0.22
Antibody-dependent cell-mediated cytotoxicity	0.22	0.33	0.17	0.65
Oxidant production	0.03	0.79	0.16	0.19
Elastase release	0.06	0.14	0.002	0.72
Cytoplasmic Ca^{2+} increase	0.01	0.001	0.001	0.003

See Table 1 for key.

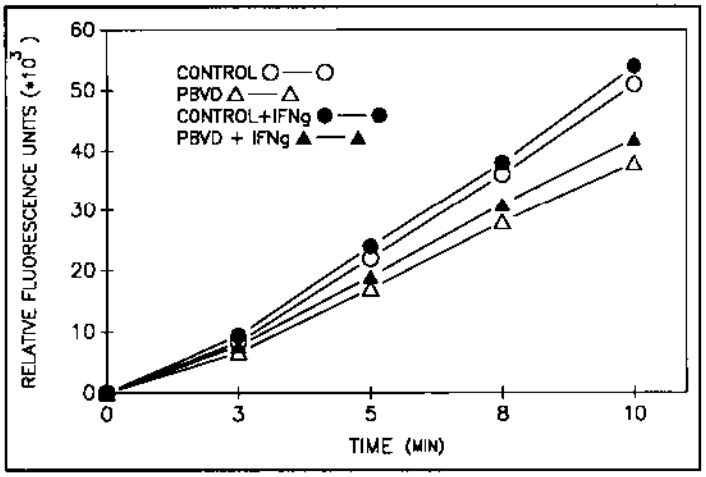

Figure 2—Mean values (n = 18) for the kinetic assay measuring elastase release from bovine neutrophils for 4 treatment groups. The mean values are the change in fluorescence units from baseline at time 0. The SEM for these mean values is 400 relative fluorescence units. *See* Figure 1 for key.

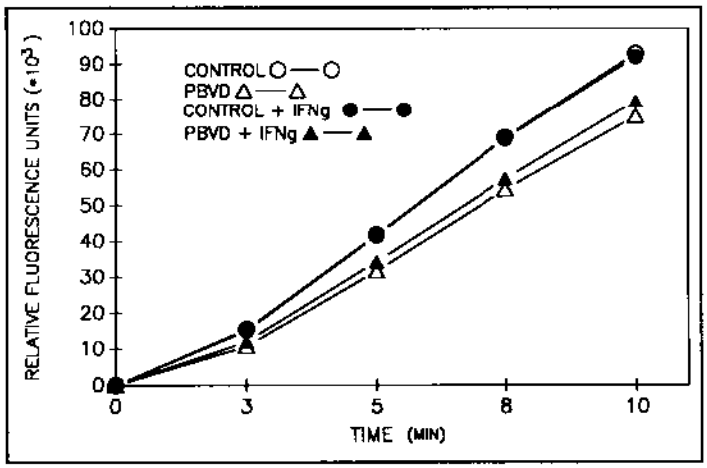

Figure 1—Mean values (n = 18) for the kinetic assay measuring oxidant production by neutrophils from 4 treatment groups. Mean values are the change in fluorescence units from baseline at time 0. The SEM for these mean values is 600 relative fluorescent units. PBVD = persistent bovine viral diarrhea; IFNG = (recombinant bovine) interferon gamma.

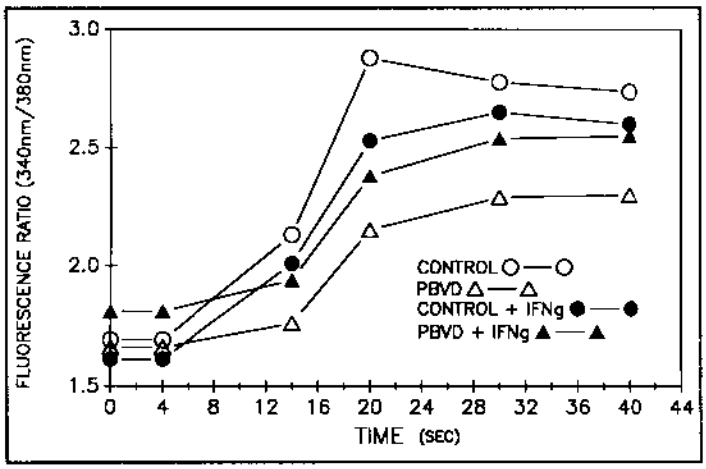

Figure 3—Mean values for the kinetic assay measuring cytoplasmic calcium fluxes in neutrophils from 4 treatment groups. Mean values represent the corrected fluorescence ratio (excitation, 340 nm/380 nm); SEM is 0.13. *See* Figure 1 for key.

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.

Table 3—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

	Treatment group assay values					Probability of > F			
Mitogen	Control (n = 17) cpm	Control + rBoIL-2 (n = 16) cpm	PBVD (n = 17) cpm	PBVD + rBoIL-2 (n = 16) cpm	SEM	Control vs PBVD	Control vs Control + rBoIL-2	PBVD vs PBVD + rBoIL-2	PBVD by rBoIL-2 interaction
None	700	4,600	500	6,600	±600	0.08	0.0001	0.0001	0.10
PHA	23,700	27,400	14,100	15,300	±2,500	0.01	0.38	0.81	0.90
ConA	49,100	54,000	21,600	22,400	±2,400	0.0001	0.35	0.91	0.91
PWM	17,300	16,600	6,000	6,600	±700	0.0001	0.64	0.67	0.29

cpm = counts per minute; PHA = phytohemagglutinin; conA = concanavalin A; PWM = pokeweed mitogen.

Discussion

This investigation extended results of a previous study[16] defining alterations of neutrophil and lymphocyte function in cattle persistently infected with BVD virus. Additional aspects of neutrophil function were found to be suppressed, and 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 BVD virus infection similarly as for cattle immunosuppressed by administration of dexamethasone.[27-28] In vitro incubation with rBoIL-2 was not able to alter depression of lymphocyte blastogenesis in cattle persistently infected with BVD 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.[29-32] Interferon gamma (in vitro and in vivo) alters neutrophil functions of cattle, although results are variable.[27,33-35] 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.[27,35] 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.[36,37] Also, protein synthesis and arachidonic acid metabolism by neutrophils are needed for IFN gamma to modulate some, but not all bovine neutrophil functions.[33]

Incubation of rBoIFN gamma with neutrophils obtained from our cattle persistently infected with BVD virus significantly enhanced AINC, elastase release, 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 BVD virus infection was detected for neutrophil chemotaxis, cytochrome-C reduction, iodination, 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 BVD virus infection.

The mechanisms for the decreased cytoplasmic calcium flux and decrease in other neutrophil functions of cattle persistently infected with BVD 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.[38] 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 cytoplasmic 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.[23,38,39] It is likely that the decreased cytoplasmic calcium flux in neutrophils from cattle persistently infected with BVD virus contributes to the 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 BVD 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 BVD virus infection.[16] Other forms of BVD virus infection also are reported to result in decreased lymphocyte blastogenic response to mitogens.[12-15,40-41] The mechanism for the decrease in blastogenesis is not known.

Differences in age, gender, breed, and husbandry were evident between the BVD virus-infected and control cattle. The possibility exists that these factors could have contributed to the differences observed between the groups. The fact that the defects in neutrophil and lymphocyte

function in these cattle persistently infected with BVD virus were similar to those described in a different group of cattle[16] 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.[42] 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[43] 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.[42,44] An additive effect on blastogenesis was seen when lymphocytes from control cattle were incubated with rBoIL-2 and either PHA or conA (Table 3), although the effect was not statistically significant. This finding was consistent with a previous report that optimal concentrations of conA and recombinant human IL-2 had an additive effect on bovine lymphocyte blastogenesis.[45] However, this additive effect was not observed in the lymphocyte cultures from persistently infected cattle, even though the rBoIL-2 significantly increased background counts. The cause for this lack of 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,[46] it may be that a similar defect in signal transduction is responsible for inhibition of lymphocyte and neutrophil functions.

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