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Lymphocyte blastogenesis and neutrophil function in cattle persistently infected with bovine viral diarrhea virus

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SUMMARY

Neutrophil function and mononuclear cell proliferative responses to mitogens were determined in healthy cattle and in cattle persistently infected with bovine viral diarrhea (BVD) virus. Uptake of [^{3}H]thymidine by resting and mitogen-stimulated peripheral blood mononuclear cells was significantly lower in cattle persistently infected with BVD virus than in healthy cattle. Neutrophils from cattle persistently infected with BVD virus had significantly impaired capability to ingest *Staphylococcus aureus*, but were normal in respect to random migration under agarose, cytochrome C reduction, iodination, and antibody-dependent cell-mediated cytotoxicity. Impairment of neutrophil function in cattle persistently infected with BVD virus differs from impairment of neutrophil function reported in healthy cattle mounting an immune response to recent BVD virus infection.

Bovine viral diarrhea (BVD) virus is ubiquitous in the cattle population. The most frequent form of BVD virus infection in cattle is a subclinical or mild disease that is characterized by transient fever, inappetence, and moderate leukopenia, with or without mild diarrhea, followed by production of BVD virus-neutralizing antibodies and rapid recovery.[1] Perhaps the most important aspect of this form of BVD virus infection is immunosuppression, which increases the host's susceptibility to secondary bacterial or viral infection. This mild form of BVD virus infection may induce spontaneous bacteremia,[2] facilitate induction of pneumonia with *Pasteurella haemolytica*,[3] allow for dissemination of infectious bovine rhinotracheitis virus infection,[4] inhibit lymphocyte blastogenesis,[2,5] decrease the number of T lymphocytes in peripheral blood,[6] and inhibit neutrophil function.[7]

A persistent form of BVD virus infection also has been reported.[8-13] If a cow is infected with noncytopathic BVD virus during the first 125 days of gestation, the virus may infect the fetus, resulting in the birth of a calf persistently infected with noncytopathic BVD virus.[13] Persistently infected calves are relatively healthy, yet have constant viremia. If an animal that is persistently infected with noncytopathic BVD virus is subsequently infected with a cytopathic BVD virus, an acute or chronic fatal disease may develop, characterized by pyrexia, anorexia, leukopenia, tenesmus, and watery diarrhea that may contain flecks of blood and mucus.[12,14] Erosions and ulcerations of the alimentary tract mucosa are the principal gross lesions found at necropsy. The complete pathogenesis of this fatal mucosal disease form of BVD virus infection remains to be determined, but persistent infection with noncytopathic BVD virus followed by infection with cytopathic BVD virus is an apparently important component. The frequency of persistent BVD virus infection in cattle is not known, but preliminary studies indicate that as much as 1% of cattle may be affected.[15] Persistently infected females produce calves that are persistently infected.[13] Therefore, persistent infection with noncytopathic BVD virus may become enzootic in a herd. As discussed, cattle not previously exposed to BVD virus will usually eliminate the virus relatively easily, but have a period of increased susceptibility to infectious disease due to impaired host defense mechanisms. Cattle persistently infected with BVD virus may be relatively healthy and apparently do not have a markedly increased susceptibility to secondary infections.[8-10] The purpose in the present report was to determine whether cattle persistently infected with BVD virus have suppression of neutrophil and lymphocyte functions similar to that reported for healthy cattle mounting an immune response to recent infection with BVD virus.

Materials and Methods

Animals—Six mixed-breed noninfected control cattle (3 castrated males and 3 females; 1 to 4 years old) and 11 mixed-breed cattle (2 castrated males and 9 females; 1 to 4 years old) persistently infected with 1 of 4 noncytopathic BVD virus isolates (NEB, 3 cattle; TGAN, 3 cattle; WVA, 3 cattle or 7443, 2 cattle[a]) were used. Persistent infection was obtained by in utero exposure to the noncytopathic BVD virus isolates as described[13] and was confirmed by repeated isolation of virus from the peripheral blood. Lymphocytes and neutrophil functions were evaluated for the 6 control cattle and for the 6 cattle infected with the NEB or the TGAN virus isolates on Feb 20 and Mar 12, 1985, and for all 17 cattle on Apr 15 and 29, 1985.

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[a] Field isolates of noncytopathic BVD virus from persistently infected cattle from Nebraska, Georgia, West Virginia, and Iowa.

TABLE 1—Values for variables of neutrophil function for healthy cattle and cattle persistently infected with bovine viral diarrhea (BVD) virus

Variable	Healthy cattle (n = 24)	Cattle persistently infected with BVD virus (n = 34)
Random migration (mm^2)	38.3 ± 3.0	36.6 ± 3.7
Staphylococcus aureus ingestion (%)	31.3 ± 3.1	20.9 ± 1.6*
Cytochrome C reduction (OD)	0.220 ± 0.013	0.196 ± 0.012
Iodination (nmol of NaI/10^7 PMN/hr)	21.2 ± 2.3	19.8 ± 1.8
Antibody-dependent cell-mediated cytotoxicity (%)	36.8 ± 4.0	38.3 ± 3.1

* Significantly different from healthy cattle ($P < 0.01$; conservative *df* were used in the F test).

Data are expressed as mean ± SEM. n = No. of blood samples evaluated from 6 healthy and 11 persistently infected cattle. OD = optical density. PMN = polymorphonuclear leukocytes.

Leukocyte isolation and functional analysis—Mononuclear leukocytes were isolated from peripheral blood (with added anticoagulant), using centrifugation on Ficoll-Hypaque,[b] and polymorphonuclear leukocytes (PMN) were isolated, using a centrifugation and flash lysis technique as described.[16,17] Lymphocyte blastogenesis in response to the mitogens phytohemagglutinin, concanavalin A, and pokeweed mitogen was done, using a microtitration procedure as described.[16] Evaluation of PMN random migration, *Staphylococcus aureus* ingestion, iodination, and antibody-dependent cell-mediated cytotoxicity were done as described.[16,17]

Cytochrome C reduction—Cytochrome C reduction by stimulated and nonstimulated neutrophils was evaluated, using a microtitration procedure. The standard reaction mixture contained 0.075 ml of cytochrome C (538 μm/ml), 0.025 ml of PMN (5.0×10^7/ml), 0.025 ml of opsonized zymosan preparation (7.5 mg/ml), and 0.05 ml of Hanks's balanced salt solution (without phenol red). Zymosan was deleted for determination of resting cytochrome C reduction. All reactants were added to a 96-well microtitration filtration plate[c] and incubated at 37 C for 30 minutes with constant agitation. The reaction was stopped by vacuum filtration and transfer of filtrate to a flat-bottom microtitration plate (Millipore vacuum holder).[c] The entrapped PMN were washed twice by filtration with 0.075 ml of Hanks's balanced salt solution which was also collected. The filtrate optical density at 550 nm was determined. Results were expressed as optical density/1.25×10^6 PMN/30 min.

Statistical analysis—An analysis of variance procedure was used to determine the level of significance of differences between healthy and persistently infected cattle. Date that the assays were done was used as a blocking factor. Conservative *df* were used (1 for each animal, rather than 1 for each blood sample evaluated).

Results

PMN function—A comparison of various aspects of PMN function in control cattle and cattle persistently infected with BVD virus is presented in Table 1. There was no significant difference between the 2 groups of cattle in the capability of their PMN to migrate under agarose, reduce cytochrome C, iodinate protein, or mediate antibody-dependent cell-mediated cytotoxicity. Persistent infection with BVD virus was associated with a marked impairment in the capability of PMN to ingest *S aureus* ($P < 0.01$).

[b] Histopaque-1077, Sigma Chemical Co, St Louis, Mo.
[c] SV, Millipore Corp, Bedford, Mass.

TABLE 2—Tritiated thymidine uptake by resting and stimulated peripheral blood mononuclear cells obtained from healthy cattle and cattle persistently infected with bovine viral diarrhea (BVD) virus

Stimulant	Healthy cattle (n = 23)	Cattle persistently infected with BVD virus (n = 32)
None	1.3 ± 0.2	0.6 ± 0.1*
Phytohemagglutinin	40.9 ± 5.0	16.2 ± 1.7*
Concanavalin A	32.3 ± 4.8	18.2 ± 3.6†
Pokeweed mitogen	15.7 ± 2.1	7.1 ± 1.2*

* Significantly different from healthy cattle ($P < 0.01$).
† Significantly different from healthy cattle ($P < 0.05$; conservative *df* were used for the F test).

Data are expressed as mean counts/min × 10^3 ± SEM. n = No. of blood samples evaluated from 6 healthy and 11 persistently infected cattle.

These results were consistent when the data were summarized by age or sex of the animal or by virus isolate.

Lymphocyte blastogenesis—Results of the blastogenesis assay for lymphocytes from control and persistently infected cattle are presented in Table 2. Incorporation of [^{3}H]thymidine by lymphocytes from cattle persistently infected with BVD virus was significantly less in both resting and stimulated lymphocyte cultures when compared with values for control cattle. Incorporation of [^{3}H]thymidine was lower for all 3 mitogens used. When the data were expressed as a stimulation index (ie, counts per minute [cpm] in cultures stimulated with mitogen divided by cpm in nonstimulated cultures), there were no significant differences between the control and persistently infected cattle, because the cpm in nonstimulated cultures (background) from persistently infected cattle was also significantly lower than that of nonstimulated cultures from control cattle. The suppression of lymphocyte blastogenesis was consistently observed when the data were summarized by age or sex of the animal or by virus isolate.

Discussion

The data presented here indicate that lymphocytes and neutrophils obtained from cattle persistently infected with BVD virus have altered function. Uptake of [^{3}H]thymidine by stimulated and nonstimulated peripheral blood mononuclear cells was inhibited, and the capability of PMN to ingest bacteria was impaired. Inhibition of mononuclear cell proliferation in persistently infected cattle is consistent with reports of the effects of BVD virus infection in cattle with a mild subclinical infection and with reports of mononuclear cell proliferation in cattle with the severe mucosal form of BVD virus infection.[2,5,18] The BVD virus has been shown to replicate in bovine lymphocytes and monocytes.[19] The direct infection of these cells with virus might impair their proliferative response.

Healthy cattle experimentally infected with BVD virus have a transient decrease in the percentage and absolute numbers of T lymphocytes in peripheral blood which may contribute to the reported decrease in mononuclear cell proliferation.[6] However, cattle persistently infected with BVD virus apparently have acceptable numbers of T lymphocytes in peripheral blood.[14] Therefore, a numerical decrease in T lymphocytes cannot be the cause for the decrease in mononuclear cell proliferation found in per-

sistently infected cattle. This does not rule out the possibility that decreased proliferation might be due to the loss of function of a subpopulation of T lymphocytes.

Markham and Ramnaraine[20] reported that cell culture cells infected with BVD virus apparently release a small molecular weight substance (which may be an arachidonic acid metabolite) that is capable of inhibiting the proliferative response of bovine peripheral blood mononuclear cells. The in vivo significance of this finding is not clear, but it is possible that this component could be responsible for the decrease in mononuclear cell proliferation which we observed and could contribute to the immunosuppression associated with BVD virus infection.

The consequences of impaired peripheral blood mononuclear cell proliferation on immune responsiveness and susceptibility to secondary infection are not known. Although persistently infected cattle produce little or no antibody against the persistent strain of BVD virus, they do produce a humoral immune response to other BVD virus isolates and to other infective agents,[13,21,22] and they have acceptable serum concentrations of protein and immunoglobulins G1, G2, and M.[10]

The PMN dysfunction reported here (inhibition of *S aureus* ingestion for cattle persistently infected with BVD virus differs from that in healthy cattle challenge exposed with virulent[7] or modified-live BVD virus.[16] In those cattle, iodination is consistently and markedly suppressed, neutrophil-mediated antibody-dependent cell-mediated cytotoxicity is significantly inhibited, and ingestion by PMN is not impaired. In fact, when ACTH was given to cattle which also were given modified-live BVD virus, the capability of PMN to ingest *S aureus* was actually increased.[16] The reasons for the differences in PMN function in persistent infection with noncytopathic BVD virus vs acute infection with cytopathic BVD virus are not known. It cannot be attributed to the cytopathogenicity of the virus, because both cytopathic and noncytopathic strains of BVD virus have been shown to inhibit PMN iodination in vivo.[7]

It may be that alteration of PMN function in healthy cattle exposed to BVD virus is due to some aspect of the host immune response. Persistently infected cattle do not mount a normal immune response and are apparently immunotolerant to the homologous BVD virus.[8] Perhaps cytokines released during a virus-induced immune response in healthy cattle could result in the alteration of neutrophil function.

The cause for the inhibition of *S aureus* ingestion by PMN from persistently infected cattle is not known. There is some evidence that immunotolerance is not complete in persistently infected cattle, and some antibody specific for BVD virus may be produced, resulting in immune complex formation.[23] Immune complexes may interfere with Fc receptor function and thereby inhibit ingestion of the *S aureus* which was opsonized with antibody.

These data indicate that both cattle persistently infected with BVD virus and healthy cattle mounting an immune response to a recent infection with BVD virus have impaired neutrophil function, but that impairment of neutrophil function in these 2 groups is different. The difference probably cannot be attributed to differences in the virus isolates involved. This implies that the alterations in neutrophil function are due to the differences in the host's response to the virus.

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