Refereed Article

Evaluation of Viral Shedding and Immune Response Following Vaccination With a Modified Live BHV1 Vaccine

James A. Roth, *DVM, PhD, Diplomate ACVM*[1]
Michael B. Vaughn, *DVM, MS*[2]
[1]*Department of Microbiology, Immunology & Preventive Medicine*
College of Veterinary Medicine, Iowa State University
Ames, Iowa 50011
[2]*Bayer Animal Health*
6750 S. Sabine Dr.
Columbia, MO 65203

Summary

The purpose of the study was to monitor Bovine Herpesvirus-1 (BHV1) shedding to susceptible herdmates after vaccination with a modified live BHV1 vaccine and to monitor the development of the cell-mediated immune response to BHV1 (as measured by antigen specific lymphocyte blastogenesis). Ten calves received a modified live virus (MLV) vaccine containing BHV1, bovine viral diarrhea virus, parainfluenza 3 virus and bovine respiratory syncytial virus. Five calves that were seronegative for BHV1 served as contact controls. Multiple attempts to isolate BHV1 from nasal swabs and buffy coat cells from vaccinates and control animals between days 0 and 21 were all negative. By six days post-vaccination five out of ten vaccinated calves had a detectable post-vaccination lymphocyte blastogenic response to BHV1 antigen. The response continued to increase until at least 28 days after vaccination, at which time all ten calves exhibited a detectable response.

None of the contact control animals developed an antibody or lymphocyte blastogenic response to BHV1 antigens. These results indicate that the MLV-BHV1 vaccine used in this study induced an early and strong cell-mediated immune response as measured by lymphocyte blastogenic responsiveness to BHV1 antigens and the vaccine virus was not shed to susceptible herdmates.

Introduction

Maximal safety and efficacy is an important goal for all vaccination programs. For Bovine Herpesvirus 1 (BHV1) vaccines maximal efficacy includes early and strong induction of cell-mediated as well as antibody-mediated immunity. Maximal safety in a herd includes no vaccine virus shedding to susceptible herdmates. Modified live vaccines are believed to generally be more effective than killed vaccines in producing rapid, longer lasting protection and for inducing cell-mediated immunity.[6] However, there has been concern that intramuscularly administered MLV-BHV1 vaccines may be shed by vaccinated animals and infect susceptible herdmates, possibly leading to abortion. The goal of this study was to determine if following vaccination with a MLV vaccine, MLV-BHV1 vaccine virus was shed to susceptible co-mingle herdmates and to monitor the development of lymphocyte blastogenic responsiveness to BHV1 antigens as a measure of a component of cell-mediated immunity.

T lymphocytes undergo a number of activities during a cell-mediated immune response to specific viral antigens. They proliferate to produce more lymphocytes that recognize the antigen, they secrete cytokines that activate various components of the immune system to control infection, and the cytotoxic T lymphocytes can directly attack and kill virus-infected cells. The lymphocyte blastogenesis assay measures the proliferative component of the cell-mediated immune response.

Materials and Methods

Animals and experimental design

Fifteen Angus x Charolais steer calves (500-600 lbs) were used in this experiment. The calves were seronegative for BHV1 and bovine viral diarrhea virus (BVDV) at the time of purchase. All 15 calves were comingled before and during the study and remained apparently healthy. After an adjustment period of two

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weeks the calves were treated daily for five consecutive days with dexamethasone (0.1 mg/kg body weight) intravenously to recrudesce any latent BHV1 infection that may be present. On the third day of dexamethasone treatment, the cattle received a single dose of oxytetracycline (Liquamycin® LA-200®, Pfizer) (9 mg/lb body weight) intramuscularly to aid in the control of any secondary bacterial infections that might occur as a result of the dexamethasone treatment. Thirty days after the start of dexamethasone treatment the calves were again tested and found to be seronegative for BHV1, indicating that none of the calves were latently infected with BHV1.

The calves were then randomly assigned to a vaccine group (n=10) and a control group (n=5). On day 0 of the study the 10 calves in the vaccine group received a 2 ml intramuscular (IM) dose of a modified live virus vaccine containing BHV1, BVDV, parainfluenza 3 and bovine respiratory syncytial virus (BRSV Vac 4, Bayer Animal Health, Shawnee Mission, KS). Following vaccination, nasal swabs and blood (buffy coats) were collected daily for eleven consecutive days and on days 13, 17, and 21 and submitted to the Iowa State University Veterinary Diagnostic Laboratory for BHV1 isolation. Blood samples were collected on days 0, 6, 10, 13, 21, 28, and 35 for lymphocyte blastogenesis assays and on days 0, 6, 9, 17, 24, 31, 41, 51, 63, and 70 for serum neutralizing antibody titers to BHV1.

Preparation of BHV1 antigens for use in lymphocyte blastogenesis assays

Stock BHV1 virus was passaged twice in Madin Darby Bovine Kidney (MDBK) cells and then used as a virus inoculum. Fresh MDBK cells were prepared in 150-cm^2 cell culture flasks (Corning) and incubated in Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 8% fetal bovine serum (FBS) and gentamicin (50 µg/ml) for 24 hours at 37°C, and then unattached virus was removed by washing. Infected cells were incubated in DMEM containing 2% FBS at 37°C in a humidified 5% CO_2 atmosphere. After a 24-hour incubation the cells were frozen at -70°C and then thawed at 37°C to release cell-associated virions. The culture media was harvested and clarified by centrifugation at 2000 rpm for 10 min. The supernatant was then collected, and virus was pelleted by ultracentrifugation at 4°C and resuspended in one tenth original volume of Hanks' balanced salt solution (GIBCO). The virus preparation was inactivated by heating to 56°C for 30 min. The BHV1 virus titer was determined by plaque assay. The virus preparation used contained 1.2 X 10^8 PFU/ml.

The protein concentration of the BHV1 preparation was determined spectrophotometrically using a bicinchonic acid based protein assay kit (Pierce Chemical Co) with bovine serum albumin as a standard. The virus preparation used had 60 µg protein/ml.

Lymphocyte blastogenesis assays

The mononuclear cells were isolated from whole blood using centrifugation, collection of the buffy coat, and a flash lysis technique to remove residual erythrocytes. This resulted in a preparation of greater than 90% mononuclear cells since the granulocytes sediment with the erythrocytes. The mononuclear leukocytes were resuspended at a concentration of 1 X 10^6 cells per ml in media containing RPMI 1640, 10% FBS and antibiotic/antimycotic solution. Two hundred microliters of this suspension were then added to microtiter wells. For this project, 25 µl each of the killed antigen and phytohemagglutinin (PHA-P) were added to the appropriate wells. Medium (25 µl of RPMI) was added to the control wells. All stimulants were run in triplicate.

The cells were cultured in 5% CO_2 at 39°C. After approximately 72 hours, the cultures were labelled with 0.04 uCi ^{3}H-thymidine per well and incubated for another 12 to 18 hours. The cells were harvested and the average counts per minute (cpm) of the triplicate wells was determined using a liquid scintillation counter.

Statistical Analysis

A Wilcoxon rank sum test was used to determine the level of significance of differences for each assay day in lymphocyte blastogenic responsiveness to BHV1 antigen and PHA.

Results

Virus Isolation

The nasal swabs and buffy coat samples were negative for BHV1 on all sampling days from all 15 calves.

Antibody Response to BHV1

The geometric mean titer for the vaccination and control groups are found in Table 1. A titer of <1:2 was defined to be zero for the purposes of calculating a geometric mean titer. All vaccinates produced a detectable antibody response with the peak geometric mean titer detected at day 31 post-vaccination. None of the contact control calves produced detectable antibody to BHV1.

Table 1. Geometric mean serum neutralizing antibody titers to BHV1 at various times after vaccination for the vaccinate and contact control calves.

Day Post-Vaccination	Vaccinates (n=10)	Contact Controls (n=5)
0	neg	neg
6	neg	neg
9	1.9	neg
17	38.0	neg
24	51.9	neg
31	53.8	neg
41	29.9	neg
51	27.9	neg
63	14.4	neg
70	15.0	neg

Lymphocyte Blastogenesis Assay

The results of the lymphocyte blastogenesis assays are presented in Tables 2 and 3. The background cpm for each treatment group are found in Table 2. The background cpm reflects the amount of tritiated thymidine uptake by unstimulated lymphocytes. Tritiated thymidine uptake is directly related to the amount of DNA synthesis in the cells.

The vaccinated calves had significantly suppressed lymphocyte blastogenic responsiveness to PHA on days 6 and 21 after vaccination when the data were analyzed as the difference in counts per minute and on day 6 when the data were analyzed as the stimulation index (Table 2).

The lymphocyte blastogenic responsiveness to BHV1 antigens is presented in Table 3. The data are

Table 2. *In vitro* blastogenic response of lymphocytes from control and vaccinated calves to phytohemagglutinin (PHA).

	Mean (±SEM Background cpm		Mean (±SEM) PHA stimulated dcpm		Mean (±SEM) PHA stimulation index	
Days Post-Vaccination	Controls (n=5)	Vaccinates (n=10)	Controls (n=5)	Vaccinates (n=10)	Controls (n=5)	Vaccinates (n=10)
0	1,140 ± 570	650 ± 270	17,780 ± 9,850	10,030 ± 2,660	17.9 ± 7.5	29.9 ± 9.8
6	720 ± 210	310 ± 99*	15,230 ± 5,810	803 ± 360**	22.1 ± 9.1	5.4 ± 2.3
10	846 ± 220	407 ± 97	12,730 ± 5,440	4,150 ± 810	15.3 ± 4.5	17.2 ± 3.8
13	3,120 ± 1,710	600 ± 180*	11,660 ± 3,580	3,630 ± 1,320	7.2 ± 1.3	7.3 ± 1.3
21	560 ± 57	390 ± 107	9,640 ± 3,640	2,100 ± 970*	19.0 ± 6.6	6.1 ± 1.0
28	1,030 ± 340	430 ± 137	10,690 ± 4,050	3,860 ± 1,230	12.5 ± 4.3	12.6 ± 3.3
35	785 ± 200	365 ± 83	10,770 ± 3,920	5,660 ± 1,660	13.2 ± 3.8	17.5 ± 3.8

cpm = counts per minute
dcpm = difference in counts per minute (stimulated cpm - background cpm)
stimulation index = stimulated cpm/background cpm
*P<0.05; **P<0.01; Level of statistical significance of the difference between the vaccinate value and the corresponding control value when analyzed by the Wilcoxon rank sum test.

Table 3. *In vitro* blastogenic response of lymphocytes from control and vaccinated calves to BHV-1 antigen.

	Mean (±SEM) Difference in counts per minute		Mean (±SEM) Stimulation Index (SI)		Number with SI greater then 3	
Days Post-Vaccination	Controls (n=5)	Vaccinates (n=10)	Controls (n=5)	Vaccinates (n=10)	Control (n=5)	Vaccinate (n=10)
0	-280 ± 368	33 ± 128	1.8 ± 1.0	1.4 ± 0.2	1/5	0/10
6	-90 ± 173	680 ± 192*	1.2 ± 0.3	4.7 ± 1.2	0/5	5/10
10	-42 ± 184	1,230 ± 230**	1.2 ± 0.3	5.0 ± 0.7**	0/5	8/10
13	-437 ± 494	4,296 ± 1,050**	1.0 ± 0.2	15.2 ± 7.0	0/5	8/10
21	6 ± 101	2,420 ± 710*	1.1 ± 0.2	12.4 ± 4.3	0/5	7/10
28	-61 ± 196	13,560 ± 2,920**	1.0 ± 0.1	57.8 ± 13.6*	0/5	10/10
35	177 ± 153	11,500 ± 3,030*	1.3 ± 0.2	57.9 ± 22.6	0/5	10/10

Difference in counts per minute = stimulated cpm - background cpm
Stimulation index = stimulated cpm/background cpm
*P<0.05; **P<0.01; Level of statistical significance of the difference between the vaccinate value and the corresponding control value when analyzed by analysis of variance.

presented as the means (±SEM) of the difference in counts per minute and the stimulation indicies (SI). For the purposes of this study, an SI greater than 3 was considered a positive response to BHV1 antigens. Table 3 also contains a summary of the number of animals in each group at each sampling time that had a BHV1 SI greater than 3. The only time that any of the control animals had a BHV1 SI greater than 3 was one animal on day 0. This is apparently an aberrant finding since the animal had a low BHV1 SI on all subsequent sampling dates. The high BHV1 SI on day 0 was due to the abnormally low background value for this animal. By six days post-vaccination five out of ten vaccinated calves had a detectable lymphocyte blastogenic response to BHV1 antigen. The response continued to increase until twenty-eight days post-vaccination, when the magnitude of the response reached its maximum and all ten calves had a detectable response.

Discussion

The study demonstrates that none of the ten vaccinated calves shed the modified live BHV1 during the period of the study. This conclusion is supported by the inability to recover the virus from nasal secretions or buffy coat cells and by the fact that the five co-mingled contact controls did not produce antibody or a detectable lymphocyte blastogenic response to BHV1 antigens. This indicates that when this BHV1 MLV vaccine is used in healthy, non-stressed cattle, shedding of the vaccine virus does not occur.

Phytohemagglutinin (PHA) was used as a non-specific mitogen in these assays (Table 2). It will stimulate T lymphocytes in a non-clonally specific manner to undergo mitosis. This causes DNA synthesis and uptake of tritiated thymidine. The cattle that received vaccine had significantly suppressed lymphocyte blastogenic responsiveness to PHA after vaccination (Table 2). This is not unexpected, as it has been previously shown that a modified-live BVD vaccine caused significant suppression of lymphocyte blastogenic responsiveness to PHA in cattle.[7] The other modified live viruses in BRSV Vac 4 may also have contributed to this suppression. Others have shown that infection with virulent BHV1 causes decreased lymphocyte blastogenic responsiveness to mitogens in cattle.[1,2,4]

The decreased lymphocyte blastogenic response to mitogens indicates a decreased ability of lymphocytes in peripheral blood to undergo mitosis for a period of time after vaccination. This may be due to a decreased functional ability of the lymphocytes or to a redistribution of the more active lymphocytes from the blood into the lymphoid tissues. The clinical significance of the decreased lymphocyte blastogenic responsiveness to PHA isn't certain but it may indicate that the cattle were immunosuppressed for a period of time after vaccination. However, the suppression of lymphocyte function, if present, must have been minor since the calves mounted an early and strong lymphocyte blastogenic response to BHV1 antigens.

The modified live vaccine used was remarkably efficient in stimulating antigen-specific lymphocyte blastogenic responsiveness in the vaccinated animals. This is considered to be a component of the cell-mediated immune response to specific BHV1 antigens.[3,5,8] The response was detectable in five out of ten vaccinates by six days post-vaccination. This was earlier than the induction of detectable serum neutralizing antibody. The lymphocyte blastogenic responsiveness continued to increase until 28 days after vaccination, at which time all ten calves had a detectable response and the magnitude of the response reached its maximum (SI=57.8). These results indicate that the cell-mediated immune response may begin to contribute to protection from infection with BHV1 as early as six days after vaccination with this MLV vaccine.

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