Mucosal and systemic antibody responses to bovine coronavirus structural proteins in experimentally challenge-exposed calves fed low or high amounts of colostral antibodies

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SUMMARY

Ten colostrum-deprived calves were assigned to 1 of 2 treatment groups (5 calves/group), and fed colostrum that had either low (normally infected cows) or high (immunized cows) antibody titers to bovine coronavirus (BCV). All calves were inoculated orally and intranasally with virulent BCV when they were 24 to 48 hours old and challenge exposed 21 days later. Blood, feces, nasal secretions, tears, saliva, and bronchoalveolar lavage (BAL) fluids were collected weekly from each calf for 5 weeks after inoculation. The titers to whole BCV or the relative amounts of isotype-specific antibodies to BCV structural proteins were evaluated in these samples by ELISA or immunoblotting, respectively.

Both pools of colostrum contained primarily IgG1, IgG2, and IgA antibodies to the E2 and E3 BCV proteins. Calves fed the high-titer colostrum had correspondingly higher amounts of passive IgG1 and IgA antibodies to whole BCV and to the E2 and E3 BCV proteins in serum, feces, and BAL fluid at postinoculation week 1 than those calves fed low-titer colostrum. Active IgG1, IgA, and IgM antibody responses in serum and active IgA and IgM antibody responses in most mucosal secretions to whole BCV and to the E2 and E3 proteins were lower or delayed in calves fed high-titer colostrum, compared with responses in calves fed low-titer colostrum. In contrast, increased responses to the BCV N protein were observed in all samples (except in serum and BAL fluid) in the calves fed high-titer colostrum, compared with calves fed low-titer colostrum. Upon challenge exposure, responses to E2 and E3 BCV proteins in serum and BAL fluid were lower in the group fed high-titer colostrum, compared with those in the group fed low-titer colostrum.

Our findings indicate that the level of passive immunity in calves at the time of BCV inoculation can influence the development of active antibody responses in serum, feces, and mucosal secretions to whole BCV and to some BCV proteins individually.

Bovine coronavirus (BCV) is a pneumoenteric virus that causes neonatal diarrhea in calves. In addition, it can infect upper respiratory tract epithelium in young and older calves under experimental and field conditions.

The virus consists of 4 major structural proteins: E1, the matrix protein, 23 to 27 kilodaltons (kD); E2, the peplomer protein, 100 to 107 kD; E3, the hemagglutinin protein, 120 to 140 kD; and N, the nucleocapsid protein, 50 to 64 kD. The E2 and E3 proteins elicit virus-neutralizing antibodies.

The active antibody responses in the intestinal and respiratory tracts to experimental infections with BCV or rotavirus in colostrum-deprived calves are primarily associated with the IgA and IgM antibody isotypes. Bovine coronavirus infection in colostrum-deprived calves elicits primarily IgG1 antibodies to the N and E2 BCV proteins in serum, but primarily IgA antibodies to all BCV proteins in feces and mucosal secretions. There may be both local antibody production at mucosal sites of viral replication and antibody production at associated systemic sites resulting in serum antibodies. Immunoglobulin A antibodies on mucosal surfaces to some or all of the BCV proteins may be important in protection from reinfection by BCV. To our knowledge, the isotype-specific antibody responses to BCV proteins in calves fed colostrum have not been described. Similar studies done in other species with other viruses dealt mainly with systemic and to a lesser extent with mucosal antibody responses.

Protection in calves against pneumonia and enteritis, by colostrum feeding, has been reported for several disease agents. Protection from mucosal infections does not always correlate well with the presence of high concentrations of systemic antibodies. Local mucosal immunity may be more important in protection and recovery from viral enteric and respiratory tract infections. Mucosal immunity is important for preventing viral, bacterial, and protozoal infections of mucosal sur-
faces; however, it is unclear how mucosal immunity is enhanced or suppressed by the presence of various levels of passive immunity. High levels of passive antibody can reduce active systemic and mucosal antibody responses to viral infections.16,18,25,31,35-37 To our knowledge, no studies have examined the BCV protein-specificity of colostral antibodies from naturally infected or immunized cows. In addition, the effect that high levels of maternal BCV antibodies, of defined specificity, have on the development of the active BCV protein-specific antibody responses in colostrum-fed calves is also undefined.

Although a maternal vaccine is commercially available for enhancement of passive protection against neonatal diarrhea caused by BCV, its efficacy is questionable.16,38,39 To develop more efficacious vaccines, a better understanding of BCV protein specificity of passive antibodies and how these antibodies influence the development of active immunity to BCV proteins in the respiratory and intestinal tracts is needed.

Therefore, our first objective of the study reported here was to characterize the isotype-specific antibody responses to BCV in serum, feces, tears, nasal secretions, saliva, and bronchoalveolar lavage (BAL) fluids after BCV inoculation and then challenge exposure in 2 groups of calves, ie, those fed colostrum containing low levels of BCV antibodies, from naturally infected cows, and those fed colostrum containing high levels of BCV antibodies, from immunized cows. Our second objective was to characterize the isotype-specific antibody responses to each of the BCV proteins (E1, E2, E3, and N) in these same samples from calves in the 2 treatment groups. In addition, we wanted to evaluate the effect of the 2 levels of passive immunity on the development of the active antibody responses reported previously.19

Materials and Methods

Colostrum pools—A. Immune colostrum pool. The colostrum pool was obtained from 3 Holstein dairy cows in the OARDC research herd, immunized with binary ethylenamine-inactivated BCV in incomplete Freund adjuvant. Cows were inoculated IM at 1 week prior to end of lactation and by intramammary infusion of BCV 1 week after end of lactation with the NCDV strain of BCV. Virus titers, amounts, and immunization procedures were as described previously.16,40-44 The virus-neutralization titer by plaque reduction assay42 to the NCDV strain of BCV in this pool of colostrum was approximately 20,000.

B. Normal colostrum pool. A pool of normal colostrum was obtained from 3 nonimmunized cows in the same herd. Commercial rotavirus/coronavirus vaccines were not used in this herd. The virus-neutralization titer by plaque reduction assay42 to the NCDV strain of BCV in this pool of colostrum was approximately 2,000. Immune and normal colostrums were collected within the first 3 milkings after calving, pooled, and stored frozen in aliquots at −20°C.

Experimental design—Experimental studies were done in 10 newborn isolation-reared colostrum-deprived calves as described previously.41 The calves were randomly assigned to 2 groups, consisting of 5 calves/group. Colostrum supplements were fed in 1 L of human infant formulaa for the first 5 to 7 days (beginning within approx 12 hours after birth) as follows: the treatment group was given 744 ± 131 ml of “immune” colostrum (120 ml/d in 2 × 60 ml or 3 × 40-ml feedings/d), whereas the control group was given 284 ± 36 ml of “normal” colostrum (40 to 60 ml/d in 2 × 20 ml or 3 × 20-ml feedings/d). Calves were maintained on human infant formulaa for the first 21 days and milk replacer for the remainder of the experiment. Calves were fed less “normal” colostrum, because less was available relative to larger amounts of the “immune” colostrum required for complete protection. Clinical signs and rectal temperatures were recorded daily.

All calves were inoculated orally and intranasally with a suspension of live virulent BCV prepared from the intestinal contents of an infected gnotobiotic calf.9 Inoculation was done when calves were between 24 and 48 hours old, with challenge exposure 21 days later.

Samples from calves—Sampling was done as described previously.19 Briefly, blood, feces, nasal secretions, tears, saliva, and BAL fluids45,46,47 were collected prior to colostrum feeding and then weekly for 5 weeks after inoculation. In the control group (calves fed “normal” colostrum), BAL fluid was collected from only 3 calves. All secretory and fecal samples collected were assessed for serum contamination by evaluating the hemoglobin content with a rapid dipstick methodb or seracult paper,c respectively. All samples, except BAL fluid and saliva, were collected uniformly and were therefore considered to be of similar concentration. Bronchoalveolar lavage fluid was standardized to 30 mg/ml by weight, as described.19

All fecal samples were examined by immunofluorescent microscopy for enteric viruses and by cell culture immunofluorescence assay for group-A rotavirus, as described previously.45 Cells from nasal swabs were processed and examined by direct immunofluorescence assay for BCV antigens, as described previously.9

Virus preparations—Virus preparations were as described previously.19 Attenuated NCDV bovine coronavirus grown in Madin-Darby bovine kidney (MDBK) cells48 was used in all immunoblots and as the coating antigen in the ELISA. Virulent BCV (DB-2 coronavirus) passaged in a gnotobiotic calf was used for calf inoculation and challenge-exposure studies.9 Previous calf challenge-exposure studies, using this inoculum orally/intranasally in gnotobiotic or colostrum-deprived calves, have shown that it induces diarrhea within 24 to 72 hours after exposure in 100% of the challenge-exposed animals and that it is free of any detectable adventitious enteric viruses or other agents.9 Hyperimmune antiserum made against this BCV inoculation pool was negative for bovine viral diarrhea virus antibodies by indirect immunofluorescence. In addition, no bovine viral diarrhea virus antigens have been found in nasal cells or intestine smears by indirect immunofluorescence from calves infected with this BCV inoculation pool.

Antibody assays—A. Enzyme-linked immunosorbent assay. An ELISA assay, as described previously,19 was used to quantify the immunoglobulin isotype titers of BCV antibodies in the “normal” and “immunee” colostrum pools.
and in serum, feces, BAL fluid, tears, nasal secretions, and saliva. Briefly, microtiter ELISA plates were coated with semipurified BCV or with mock-infected cell culture fluids (control) prepared identically as the virus material. Reagents were added as follows: primary antibody to be measured—fourfold dilutions of samples (starting at 1/25 for feces and nasal swab fluid and 1/16 for all other samples) added in duplicate to virus and control wells, plus a positive and negative serum standard per plate; secondary antibody—optimal diluted monoclonal antibodies to bovine IgG1, IgG2, IgA, and IgM (heavy chain-specific); indicator antibody—optically diluted goat anti-mouse IgG (H + L chains) conjugated to the enzyme horseradish peroxidase; and the substrate 1 mM 2,2-azino-bis-(3 ethylbenzthiazoline sulfonic acid) plus 0.1% H₂O₂ in 0.1M citrate buffer, pH 4.0. The criteria for positive absorbance values in ELISA were as described previously.

The mean antibody titer (log₁₀) from the 5 calves was determined for each sample type and the 4 isotypes. To determine whether passive transudation or selective transfer of antibody isotopes from serum to secretions was occurring, the ratios of IgG1/IgG2 and IgG1/IgA titers were calculated for each sample type and time point and were averaged. Samples that were negative at the lowest dilution tested were assigned a value of one-half the log₁₀ of the reciprocal of the lowest dilution tested. For example, if a sample was negative at the lowest dilution tested of 1.2 (log₁₀), then it would be assigned a value of 0.6 (log₁₀).

B. Immunoblot assay. An immunoblot assay, as described previously was used to determine the antibody isotype viral protein-specific antibody responses in the "normal" and "immune" colostrum pools and in serum, feces, BAL fluid, tears, and nasal secretions collected from inoculated and challenge exposed calves. Briefly, the 4 major structural BCV proteins (E1, E2, E3, and N) from cell culture grown virus, were separated by electrophoresis, transferred to nitrocellulose paper, blocked, and reacted with 15 samples in separate lanes for 2 hours. Samples were tested as follows: serum at a dilution of 1:10; fecal samples at a dilution of 1:25 (w/v); nasal swab fluids as collected in cell culture medium; and BAL fluid and lacrimal secretions at a dilution of 1:4 after processing as described. The isotypes of antibodies to BCV proteins were detected by reaction with isotype-specific monoclonal antibodies (heavy chain-specific). Localization of bound antibody was achieved with anti-mouse Ig (H+L chains) conjugated to horseradish peroxidase followed by the chromogenic substrate, tetramethylbenzidine with H₂O₂. The strength of the immune response was visually assessed by grading the intensity of the staining reaction from 0 (no reaction), +1 (weak reaction), +2 (moderate reaction) and +3 (intense reaction) for each protein. Any amount of nonspecific binding (reaction intensity on preexposure samples) was taken into account when evaluating the reaction intensity of the postexposure samples. Nonspecific reaction intensities were considered background and mathematically subtracted from the value given to the degree of sample binding in

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* Immulon 1, Dynatech Laboratories Inc, Chantilly, Va.
* Supplied by Dr. Srikumaran, University of Nebraska, Lincoln, and Dr. Goldsby, Amherst College, Amherst, Mass.
* Boehringer Mannheim Biochemicals, Indianapolis, Ind.
* Sigma Chemical Co, St Louis, Mo.

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postexposure samples, except in the evaluation of tears, and BAL fluid in which no precolostrum feeding samples were taken. The grades for each isotype-specific protein-specific response within a sample type and time point were averaged for the 5 calves.

Statistical analysis—A one-way analysis of variance was used to evaluate statistically significant differences in mean antibody titers between the control and treatment groups for each isotype at each time point. An F test was used to determine whether differences in the means were significantly different at a level of 0.05.49,50

Results

Clinical signs and samples—A. Treatment group. After inoculation, 4 of 5 calves had BCV-positive nasal epithelial cells by immunofluorescence, without clinical signs of rhinitis. After challenge exposure, 4 of 5 calves (not the same 4 calves) also had BCV-positive nasal epithelial cells by immunofluorescence, without rhinitis. The immunofluorescence seen was similar to that observed in our previous study.9

During the period of colostrum feeding, no calves shed BCV in feces (detected by IEM) but 3 of 5 calves shed BCV in feces associated with diarrhea during the period after colostrum feeding. One of 5 calves shed BCV in feces without diarrhea after challenge exposure. Three of 5 calves had rotavirus infections, 2 at postinoculation days (PID) 10 to 13 and 1 at PID 21 to 22 (as determined by cell culture immunofluorescence), all associated with diarrhea. Two calves also shed small round structured viruses in their feces at PID 5 (as determined by IEM) unassociated with diarrhea. These small round structured viruses are unclassified as of yet, and are categorized only on the basis of morphologic features by electron microscopy.

B. Control group results. After inoculation, all 5 calves had BCV-positive nasal epithelial cells by immunofluorescence, without clinical signs of rhinitis. After challenge exposure, only 1 of 5 calves had BCV-positive nasal epithelial cells by immunofluorescence, without clinical signs of rhinitis. All calves shed BCV in feces (as detected by IEM) associated with diarrhea during and after the period of colostrum feeding, but none shed BCV in feces after challenge exposure. One of 5 calves had a rotavirus infection in the second week of the study (as determined by cell culture immunofluorescence) and 2 calves also shed small round structured viruses in their feces at postinoculation days 0 to 4 (as determined by IEM).

In both treatment and control groups, all fecal and most mucosal secretion samples (except saliva) were free of hemoglobin and were therefore considered to be free of serum contamination. Some BAL fluid samples contained trace amounts of hemoglobin, indicating some leakage of serum into the sample. All saliva samples were contaminated with variable amounts of hemoglobin and were, therefore, not evaluated in the immunoblot assay, but were tested in the ELISA.

Enzyme-linked immunosorbent assay—The ELISA antibody isotype titers (log_{10}) in the “normal” colostrum for IgG1, IgG2, IgA, and IgM were 4.4, 4.0, 2.6, and undetectable, respectively. Antibody isotype titers in “immune” colostrum for IgG1, IgG2, IgA, and IgM were 6.0, 4.7, 4.1, and undetectable, respectively. These differences

Immunoglobulin G1 antibody titers in the control group taken prior to colostrum feeding were below the lowest assay level. In all sample types were low at PIW 1, but then increased only for IgG1. All isotype antibody titers for all samples (P < 0.05) different between the treatment and control groups were mostly observed in the first few weeks (Fig 1). Significant differences were not observed in the isotype antibody titers in samples taken from calves that had BCV infection alone or those that remained the same in nasal secretions, feces, and tears (Fig 1). Significant (P < 0.05) differences in antibody titers between the treatment and control groups were mostly observed in the first few weeks (Fig 1).

The IgG2 antibody titers in the treatment group in all sample types (except serum and tears) were high at PIW 1, and then decreased over the study period. The IgG2 antibody titers in the control group in all sample types were low at PIW 1, and remained low in most sample types except serum, which showed a slight increase (Fig 2). Sign-

Table 1—Bovine coronavirus isotype-specific antibody titer ratios in serum, feces, and various mucosal secretions of calves in the control and treatment groups. Calves were inoculated at 24 to 36 hours after birth and challenge exposed at postinoculation week 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio</th>
<th>Control group</th>
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<td></td>
<td></td>
<td>1  2  3  4  5</td>
<td>1  2  3  4  5</td>
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<tr>
<td>Serum</td>
<td>IgG1/IgG2</td>
<td>1.2  4.9  4.7  4.8  5.3</td>
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<td>IgG1/IgA</td>
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<td>IgG1/IgA</td>
<td>1.5  0.2  0.4  0.5  0.8</td>
<td>1.7  1.3  0.6  0.8  0.9</td>
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<td>Tears</td>
<td>IgG1/IgG2</td>
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<td>IgG1/IgA</td>
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<td>IgG1/IgA</td>
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<td>3.1  2.2  2.7  1.4  0.8</td>
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<td>Saliva</td>
<td>IgG1/IgG2</td>
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<tr>
<td></td>
<td>IgG1/IgA</td>
<td>1.8  0.8  1.2  1.5  1.3</td>
<td>1.8  1.2  1.2  1.2  1.2</td>
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</table>

UD = Undetermined because numerator and denominator were both below the lowest assay level.

Figure 5—Immunoblot of the “normal” and “immune” colostrums diluted 1:16. Lane designations are as follows: lane P — hyperimmune polyclonal antiserum diluted 1:50; lanes marked N — “normal” colostrum; lanes marked I — “immune” colostrum; lane C — negative control gnotobiotic calf serum devoid of scv antibodies diluted 1:10. Lanes were reacted with anti-bovine IgG1, IgG2, IgA, or IgM monoclonal antibodies as indicated, then with goat anti-mouse serum conjugated to horseradish peroxidase, followed by the substrate tetramethylbenzidine. The left margin indicates the position of each of the 4 scv structural proteins and the right margin indicates their relative molecular masses.

Figure 6—Immunoblot reaction intensities of serum, feces, and the various mucosal secretions to the E1 BCV protein at postinoculation (PI) weeks 1, 2, 3, and the average of weeks 4 and 5 in the control (A) and the treatment (B) groups of calves. I = time of inoculation, C = time of challenge exposure. Reaction intensities graded from 0 (no reaction) to +3 (strong reaction).
nificant \( P < 0.05 \) differences in antibody titers between the treatment and control groups were again mostly observed in the first few weeks (Fig 2).

The IgA antibody titers in the treatment group were low at PIW 1 in most sample types (except in feces) and then increased slightly in nasal secretions, tears, and BAL fluid. The IgA antibody titers in the control group were also low at PIW 1 in all sample types, but then increased to significantly \( P < 0.05 \) higher levels, than in the treatment group, in all sample types (Fig 3).

The IgM antibody titers in the treatment group were low at PIW 1 in most sample types (except in feces) and then increased slightly in serum, nasal secretions, feces, and tears. The IgM antibody titers in the control group were not significantly different from those of the treatment group at PIW 1 in all sample types (except in BAL fluid). The IgM antibody titers in the control group then increased rapidly (in most sample types, except saliva) and then decreased. The peak titers were only significantly \( P < 0.05 \) different from those of the treatment group in serum (Fig 4).

In serum, IgG1/IgG2 ratios in the control group were greater than in the treatment group at most time points in the study (Table 1). However, there were greater IgG1/IgA ratios in serum in the treatment group than in the control group initially, but the difference decreased over the course of the study. In addition, IgG1/IgA ratios in nasal secretions, feces, and tears were lower at all time points in the control group vs the treatment group. However, in BAL fluid and saliva, the IgG1/IgA ratios increased at most time points in the control group, but decreased in the treatment group (Table 1).

In the control group, IgG1/IgG2 ratios were higher at most times in serum than in feces or any of the secretions. In contrast, IgG1/IgG2 ratios in the treatment group were approximately equal in serum, feces, and mucosal secretions (except in tears at PIW 1 and 2).

Because of the variable serum contamination of saliva, the ELISA antibody titers shown may not be entirely of salivary origin. However, contribution of serum antibodies appears to be minimal, as seen from a comparison of the IgG1/IgG2 ratios in serum and saliva at each time point (Table 1). However, IgG1/IgG2 ratios of BAL fluid titers in the control group approached those found in serum at PIW 3 and 4, possibly indicating serum leakage into BAL fluid.

Immunoblot analysis—Immunoblot analysis of the "normal" and "immune" colostrums revealed that both colostrums contained predominantly IgG, IgG2, and IgA antibodies to mainly the E2 and E3 BCV proteins. Although the "immune" colostrum contained higher levels
ANTIBODY RESPONSES TO N PROTEIN

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Serum</th>
<th>Fecal</th>
<th>Nasal</th>
<th>Tears</th>
<th>BAL</th>
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</table>

A NORMAL COLOSTRUM FED CALVES

B IMMUNE COLOSTRUM FED CALVES

Figure 9—Immunoblot reaction intensities of serum, feces, and the various mucosal secretions to the N BCV protein at postinoculation (PI) weeks 1, 2, 3, and the average of weeks 4 and 5 in the control (A) and the treatment group of calves (B). I = time of inoculation, C = time of challenge exposure. Reaction intensities graded from 0 (no reaction) to +3 (strong reaction).

In addition, there were some IgG1 antibodies to the E1 protein in the “immune” colostrum and some IgA antibodies to the E1 and N proteins in both colostrums.

E1 BOVINE CORONAVIRUS PROTEIN REACTIVITIES

At PIW 1, antibodies were detected to the E1 BCV protein in tears (all isotypes) and feces (IgA and IgM) in the treatment group, but not in the control group (except IgM in tears, Fig 6). At PIW 2 and 3, slightly greater antibody reactivities were observed in serum and BAL fluid from the control group than in the treatment group. Challenge exposure did not cause marked increases in any antibody isotype in any sample tested (Fig 6).

E2 BOVINE CORONAVIRUS PROTEIN REACTIVITIES

At PIW 1, IgG1 antibodies to the E2 BCV protein were detected in serum, feces, and BAL fluid; IgG antibodies in serum, feces, tears, and BAL fluid; and IgM antibodies in only feces in the treatment group, but not the control group (Fig 7). At PIW 2, IgA and IgM antibodies were detected in serum, feces, tears, and BAL fluid in the control group, but not in the treatment group (except IgA in serum and feces). In addition, actively produced IgG1 was observed in serum and tears in the control group. At PIW 3, IgA antibodies were detected in feces and all mucosal secretions in the treatment group. Immunoglobulin A antibodies were still in the serum, tears, and BAL fluid in the control group. After challenge exposure, greater antibody responses in serum (all isotypes) and in BAL fluid (IgA) were noticed in the control group than in the treatment group. However, greater IgA antibody responses were observed in feces and nasal secretions in the treatment group than in the control group (Fig 7).

Discussion

Our findings indicate that the level of passive immunity in calves at the time of BCV inoculation can influence the development of active antibody responses in serum, feces, and mucosal secretions to some BCV proteins. In our previous study,19 we showed that BCV infection in colostrum-deprived calves elicits primarily IgG1 antibodies to the N and E2 BCV proteins in serum and IgA antibodies to all BCV proteins in feces and mucosal secretions.

Both “immune” and “normal” colostrums contained antibodies (primarily IgG1, IgG2, and IgA) directed mainly toward the E2 and E3 BCV proteins, the only major difference being the higher level of these antibodies in the “immune” colostrum, as indicated by ELISA titers and the reaction intensities in the immunoblot assay (Fig 5). This indicates that immunization of the dam via the method described can induce antibody isotypes with similar protein specificity as those induced after natural infection, only in higher amounts.

Analysis of the ELISA antibody titers at PIW 1 indicated that the titers of passive IgG1 and IgG2 antibodies in serum, feces, and some mucosal secretions were signif-
tantly higher in the treatment group than in the control group (Fig 1 to 4). These higher titers of serum IgG1 and IgG2 antibodies to BCV were also reflected as higher titers of IgG1 and IgG2 antibodies in nasal secretions, BAL fluid, and saliva. These findings agree with those of Pospisil et al. who indicated that passive IgG1 appears in respiratory tract secretions. Feces from the treatment group also showed higher titers of IgG1, IgG2, and IgA antibodies at PIW 1; however, these higher titers may be from residual colostral antibodies in the digestive tract, because the calves continued receiving daily colostrum feedings through PIW 1.

From this study it cannot be determined conclusively whether higher titers of antibody isotypes seen in secretions of the treatment group over those of the control group (early in the study) were attributable to passive transudation or selective transfer from serum. We attempted to answer this question by evaluating the IgG1/IgG2 antibody titer ratios in serum and each secretion, assuming that the ratios would be similar if passive transudation were predominant. This was true in all secretions, except tears from the treatment group in which the ratio was higher than serum (at PIW 1 to 3), indicating possible selective transfer of IgG1 into tears. In addition, the higher IgG1/IgA antibody titer ratios in tears and BAL fluid, over those of feces or other secretions in the treatment group, may also indicate selective transport of IgG1 into tears and BAL fluid (Table 1). Immunoblot analysis showed that these high titers of IgG1 and IgG2 passive antibodies in serum and some mucosal secretions were specific mainly for the E2 and E3 BCV proteins. This was as expected because the colostrum received by these calves in the treatment group contained principally antibodies to the E2 and E3 BCV proteins.

Overall, the antibody responses in both groups of calves to BCV inoculation, as determined by ELISA and immunoblotting, were lower than those reported previously for inoculation of colostrum-deprived calves. Depression of active antibody responses in serum and mucosal secretions to BCV infection appears to be directly related to the level of passive antibody at the time of infection. This agrees with other studies that show high levels of passive antibody can reduce both the active systemic and local immune responses to other viruses. Active antibody iso-type responses to the whole virion (as determined by ELISA) were lower in serum, feces, and all secretions with most isotypes in the treatment group; however, in some sample types it could not be determined whether depression of the active responses had occurred because of the simultaneous presence of passive antibodies. The level of IgG1 was consistently lower in all sample types from calves in the treatment group, whereas the other isotypes were decreased in some, but not all, sample types (Fig 1 to 4). Of all isotypes, IgM seemed least susceptible to depression by maternal antibodies. This may suggest that BCV-specific IgM antibodies are suitable when seroconversion is used as the basis for making a diagnosis of BCV infection in young calves that may still have some level of passive antibody to BCV.

On challenge exposure, amounts (as determined by ELISA) of most antibody isotypes in most samples were not markedly increased in either group of calves. In contrast, colostrum-deprived calves had marked increase in IgA antibodies in several mucosal secretions. Immunosuppression of antibody responses in colostrum-fed calves as old as 10 weeks have been reported for bovine respiratory syncytial virus. Therefore, the lower responses may be caused by colostrum feeding preventing or delaying an anamnestic response (because of a suppressive effect of colostrum), or preventing BCV replication in the intestinal tract, or both.

Immunoblot analysis of the antibody responses indicated the depression to be specific to only some BCV proteins. The greatest depression (or delay) was seen in IgA and IgM responses to E3 and E2 BCV proteins. The E2 protein IgA antibody responses were delayed in serum, feces, and all mucosal secretions, and IgG2 antibody responses were depressed in serum in the treatment group, compared with the control group (Fig 7). The E3 protein IgA antibody responses were depressed in serum, feces, tears, and BAL fluid (Fig 8). In contrast, N protein antibody responses were not depressed (or delayed) in feces, nasal secretions, or tears. However, there was a depression and delay of IgA antibody responses in BAL fluid and a delay in IgG1 and IgM antibody responses in serum to the N protein in the treatment group, compared with the control group (Fig 9).

On challenge exposure, responses to all the E2 and E3 BCV proteins in serum and BAL fluid were still depressed in the treatment group, compared with those in the control group. However, on challenge exposure, antibody responses to the N protein were increased in some sample types for some isotypes, compared with those in the control group.

Calves in the treatment group had high levels of IgG1 passive antibodies to the E2 and E3 BCV proteins, which may have selectively depressed or delayed the active antibody response to these BCV proteins. Because there were only low levels of antibodies to the N and E1 BCV proteins in calves in the treatment group, the same degree of depression of the active antibody response might not be expected, as seen for the N protein. An alternative explanation may be that high levels of passive or lactogenic antibodies prevented BCV replication in the intestine (as indicated by the comparative delay of BCV shedding in the feces of the treatment group vs the control group), and, therefore, antigen stimulation by all but the most immunogenic BCV protein (the N protein). In our previous study, the N protein elicited the greatest antibody response. In addition, nonimmunoglobulin factors in colostrum have also been shown to be immunosuppressive. It has been suggested that this type of immunosuppression may be attributable to the presence of nonspecific immunosuppressive factors in colostrum, which suppress both humoral and cellular immune responses. Results from this study may support this, but also indicate a more specific mechanism of selective protein antibody depression, on the basis of a negative feedback type model, as shown by others. Some depression of the antibody response to the E1 protein was also seen, which may also have been attributable to higher levels of E1 passive antibodies in the treatment group, because there were greater levels of E1 antibodies in the “immune” colostrum, compared with the “normal” colostrum (Fig 5).

Further research is needed to determine the mechanism(s) involved in depression of active antibody responses in suckled calves and the role that these antibodies have in prevention of BCV infections in calves in the field.
References


