Monoclonal Antibodies to Bovine Coronavirus Glycoproteins E2 and E3: Demonstration of \textit{in vivo} Virus-neutralizing Activity

By DIRK DEREGT,\(^1\)† GLEN A. GIFFORD,\(^2\) M. KHALID IJAZ,\(^3\) TRENT C. WATTS,\(^3\) JAMES E. GILCHRIST,\(^2\) DEBORAH M. HAINES\(^1\) and LORNE A. BABIUK\(^1,\)\(^3\)*

\(^1\) Department of Veterinary Microbiology, University of Saskatchewan, \(^2\) Biostar, Inc., P.O. Box 1000, Sub P.O. 6 and \(^3\) Veterinary Infectious Disease Organization, 124 Veterinary Road, Saskatoon, Saskatchewan, Canada, S7N 0W0

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**SUMMARY**

Six monoclonal antibodies (MAbs) to bovine coronavirus (BCV, Quebec isolate) E2 and E3 glycoproteins which were found previously to be neutralizing \textit{in vitro} were examined for virus-neutralizing activity \textit{in vivo}. Surgically ligated intestinal loops of newborn colostrum-deprived calves were virus-inoculated, mock-infected or inoculated with a mixture of virus and antibody. Of the six BCV-specific MAbs, four were found to be protective against a virulent field isolate of BCV, as indicated by a reduction in villous atrophy. These MAbs were specific to antigenic domain A and antigenic domains A1 and A2 on the E2 and E3 glycoproteins respectively. MAbs to antigenic domains B and C on the E2 and E3 glycoproteins, respectively, were not protective.

Bovine coronavirus (BCV) is considered to be one of the leading causative agents of viral enteritis in newborn calves (Babiuk et al., 1985). The disease is characterized by diarrhoea and severe dehydration and is often fatal (Mebus, 1978; Mebus et al., 1973). BCV infects epithelial cells of the jejunum, ileum and colon resulting in severe shortening of villi (Mebus et al., 1973). The virion is composed of four structural proteins, a nucleocapsid (N) protein and three envelope glycoproteins that have been designated E1, E2 and E3 (Storz et al., 1981; King & Brian, 1982; Deregt et al., 1987). Monoclonal antibodies (MAbs) to the Quebec isolate of BCV have been produced and virus-neutralizing \textit{(in vitro)} antibodies were found to be directed to the E2 glycoprotein (gp190/gp100) and the disulphide-linked dimer glycoprotein E3 (gp124) (Deregt & Babiuk, 1987).

Our interest in the possible employment of subunit or synthetic oligopeptide vaccines for the prevention of coronavirus-induced neonatal calf diarrhoea has led us to examine the roles that the E2 and E3 proteins may play in an \textit{in vivo} infection. Specifically, we were interested in determining whether MAbs to specific epitopes on these proteins could protect calves from BCV-induced intestinal villous atrophy. Thus, to determine whether anti-BCV MAbs that were neutralizing \textit{in vitro} could also be effective against BCV infection \textit{in vivo}, six MAbs representing five antigenic groups (Table 1) were mixed with virus and inoculated into surgically ligated intestinal loops of newborn calves.

Colostrum-deprived Holstein calves were obtained from local dairy farms within a few h of birth. After transport, the calves were fed 2 l of a balanced electrolyte solution (Ionalyte; Rogar/STB) via an oesophageal feeder. Feeding was repeated 8 h later. Approximately 24 h after birth, the calves were anaesthetized by inhalation of a mixture of halothane, nitrous oxide and oxygen. Gentamicin sulphate (Garasol, Schering Canada) (50 mg) was added to 4.5 l of lactated...
Table 1. *Protective effects of BCV MAbs*

<table>
<thead>
<tr>
<th>Antibody designation</th>
<th>Isotype</th>
<th>Protein specificity</th>
<th>Antigenic group</th>
<th>In vitro neutralization titre*</th>
<th>In vivo protection†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB10-4</td>
<td>G1</td>
<td>E2</td>
<td>A</td>
<td>12500</td>
<td>+</td>
</tr>
<tr>
<td>JBS-6</td>
<td>G2a</td>
<td>E2</td>
<td>A</td>
<td>50000</td>
<td>+</td>
</tr>
<tr>
<td>BB7-14</td>
<td>G2b</td>
<td>E2</td>
<td>B</td>
<td>80000</td>
<td>−</td>
</tr>
<tr>
<td>HC10-5</td>
<td>G2a</td>
<td>E3</td>
<td>A1</td>
<td>5000</td>
<td>+</td>
</tr>
<tr>
<td>KD9-40</td>
<td>G2a</td>
<td>E3</td>
<td>A2</td>
<td>8000</td>
<td>+</td>
</tr>
<tr>
<td>BD9-8C</td>
<td>G2a</td>
<td>E3</td>
<td>C</td>
<td>800</td>
<td>−</td>
</tr>
<tr>
<td>BCV antiserum‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND§</td>
</tr>
</tbody>
</table>

*Reciprocal of the dilution of antibody (ascites fluid) that produced a 50% reduction of virus plaque number. Antibody titres were in general lower than previously reported (Deregt & Babiuk, 1987), because different ascites fluids were used in this study.

†Protection was determined by reduction of virus-induced villous atrophy in intestinal loops: +, protective; −, not protective.

‡Antiserum was obtained from a calf that survived an experimental infection with BCV isolate no. 77 and which was subsequently immunized twice with purified BCV (Quebec isolate).

§ND, Not done. ELISA titre was 512000.
were severely shortened with a mean villus length of only 135 ± 21 μm (Fig. 1). Further, BCV antigen could be detected within intestinal cells from virus-inoculated control loops, indicating that these cells were infected with virus (Fig. 2d).

To determine whether anti-BCV MAbs that had been previously found to be neutralizing in vitro could also neutralize BCV infectivity in vivo, virus inoculum was briefly mixed with MAbs and injected into intestinal loops, and the results were compared with virus-inoculated and mock-infected intestinal loops. Further, BCV polyclonal antiserum and anti-BHV-1 MAbs were also mixed with virus and injected into intestinal loops to serve as controls. Four of the six BCV-specific MAbs, E2-specific HB10-4 and JB5-6 (both antigenic group A) and E3-specific HC10-5 (group A1) and KD9-40 (group A2) were found to be protective, as indicated by the villus lengths of intestinal loops after treatment with these antibodies (Fig. 1 and 2c). Villus lengths in the intestinal loops treated with virus and these four MAbs were over 85% of those in the corresponding mock-infected loops, whereas in unprotected loops they were less than 50% of the corresponding mock-infected loops. The protective effect of BCV polyclonal antiserum was similar to that of protective MAbs (Fig. 1). After these treatments tissues showed little, if any, immunohistochemical staining for BCV antigen (not shown). In contrast, BHV-1-specific MAbs as expected, were not protective (Fig. 1 and 2b, d). E2-specific MAb BB7-14 (group B) and E3-specific MAb BD9-8C (group C) were also not protective, as the pathological changes (necrosis and villous atrophy) observed in these treatments were similar to those of virus-inoculated control loops (virus plus MEM or virus plus BHV-1-specific MAb). Further, villus length measurements from loops inoculated with a mixture of virus and these MAbs were not statistically different from virus-inoculated control loops (Fig. 1).
The amount of fluid accumulated in intestinal loops showed a positive correlation with the degree of villous atrophy ($r = 0.827$). Fluid accumulation was unexpected since it is generally thought that viral infections do not result in fluid movement into the lumen (reviewed in Babiuk et al., 1985). The protection afforded by MAb administration was also demonstrated when the amount of cell-free antigen present in the luminal contents of intestinal loops was determined by ELISA (not shown). Intestinal contents of all virus-inoculated control loops contained significant quantities of coronavirus antigen. In contrast, test loops inoculated with BCV and the MAbs that induced protection as measured by a significant reduction of villous atrophy and fluid exudation did not contain detectable levels of cell-free antigen in the fluids, with the exception of one loop where a low level of virus antigen was present.

The results show that E2-specific MAbs of antigenic group A and E3-specific MAbs of antigenic groups A1 and A2 were similar to BCV hyperimmune antiserum in protecting intestinal villi from the effects of BCV infection. These results suggest that specific amino acid sequences present on both E2 and E3 BCV glycoproteins can be potential targets for synthetic oligopeptide vaccines and support the suggestion that the E3 protein has an important biological function in BCV infectivity (Deregt & Babiuk, 1987).

Of several possible explanations for the finding that two of the six MAbs in this study neutralized virus in vitro yet were not protective in vivo, one is that there may be a lack of conservation of epitopes on the virulent isolate employed. This was apparently the case for the epitope recognized by the E2-specific MAb, BB7-14, as later antibody-binding studies showed that this MAb did not bind the challenge virus in an ELISA. In contrast, the E3-specific MAb BD9-8C bound to this virus, indicating that the epitope recognized by this MAb was conserved on the virulent isolate (not shown). Thus it is possible that epitope C on the E3 glycoprotein may be irrelevant for the infectivity of the virus in vivo. Further, the virus used in this study was exposed to antibody for only a short time, compared to in vitro studies (1 h), before it came in contact with cells. Thus, a possible difference in the binding kinetics or avidity of MAb BD9-8C may explain the difference in neutralizing activity in vitro and in vivo. Finally, since BD9-8C showed the lowest in vitro neutralizing titre of all MAbs used, the challenge dose used may have exceeded its protective capacity.

E2-specific MAbs have previously been shown to protect against infection in vivo by another coronavirus, mouse hepatitis virus (Talbot et al., 1984; Buchmeier et al., 1984; Wege et al., 1984). However, the E3 glycoprotein, identified as the haemagglutinin protein (King et al., 1985) appears to be unique to haemagglutinating mammalian coronaviruses (Hogue et al., 1984). The protective effect of two E3-specific MAbs in vivo in this study emphasizes the importance of the E3 protein in BCV-cell interactions previously indicated by in vitro virus neutralization studies.
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Fig. 2. The reduction of virus-induced pathological changes to villi by a representative anti-BCV MAb. (a, b, c) Light micrographs taken from tissues from intestinal loops. Tissues were fixed in Bouin's solution and stained with haematoxylin and eosin. These tissues were collected from loops of the same series in the lower jejunum of calf 87-015. Treatments were as follows: (a) mock-infected; (b) virus plus anti-BHV-1 MAb; (c) virus plus E3-specific MAb KD9-40. (d) Immunohistochemical localization of BCV antigen in intestinal epithelium from a control loop (virus plus anti-BHV-1 MAb). For immunochemical staining, Bouin's fixed tissues were briefly digested with 0.1 ~ protease (Type XIV; Sigma), treated with 0.15~ H202 in methanol to inactivate endogenous peroxidase and blocked with 5~ normal rabbit serum in Tris-buffered saline (TBS) before incubation with a mixture of anti-BCV MAbs. After subsequent incubation with biotin-labelled antiserum to mouse IgG, tissue sections were incubated with avidin–biotin–peroxidase solution (Vectastain ABC; Vector Laboratories). Tissues were stained by incubation in 1 mg/ml of 3,3'–diaminobenzidine (Electron Microscopic Products) in TBS and 0.5% H2O2 and subsequently counterstained with haematoxylin. All bar markers represent 200 μm.
REFERENCES


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