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Antigenic Differences between a Field Isolate and Vaccine Strains of Bovine Viral Diarrhea Virus†

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Antigenic diversity among the bovine viral diarrhea virus (BVDV) cytopathic strains 87-2552 (field isolate) and NADL and Singer (prototype strains) was demonstrated with monoclonal antibodies (MAbs) in enzyme-linked immunosorbent, immunofluorescence, virus neutralization, and immunoprecipitation assays. Two MAbs against BVDV strain 87-2552, designated D11 and B7, strongly neutralized this field strain and were specific for the 48-kDa glycoprotein of the virus. These two MAbs have different subisotypes, immunoglobulin G1 for D11 and immunoglobulin G3 for B7. MAbs against BVDV strains 87-2552 and NADL were specific for their respective strains in virus neutralization assays. The results indicated significant antigenic differences between BVDV strain 87-2552 and the NADL and Singer strains.

Bovine viral diarrhea virus (BVDV) is the causative agent of viral diarrhea-mucosal disease in cattle, an economically important disease of worldwide distribution (11). BVDV is a positive-stranded and enveloped RNA virus and a member of the genus *Pestivirus* in the family *Togaviridae*, although recent studies have suggested that it should be a member of the genus *Flavivirus* in the family *Flaviviridae* (5, 18, 20). Monoclonal antibodies (MAbs) are specific for a single antigenic determinant of viral protein and thus provide a useful approach to differentiate various strains in a virus system (12). Several reports have revealed the presence of antigenic variations in BVDV strains detected by using neutralizing MAbs (7–10, 21).

Our previous studies with a panel of MAbs raised against the NADL strain of BVDV and specific for gp53 showed antigenic variations among BVDV strains (21). One cytopathic BVDV field strain, designated 87-2552 (21), was not neutralized by any MAb. The objective of this study was to further delineate antigenic differences between this field isolate and vaccine strains (NADL and Singer).

MATERIALS AND METHODS

Virus strains and cells. The NADL, Singer, and 87-2552 strains of BVDV were obtained as mentioned previously (21). Field strain 87-2552 was isolated from a lung of an infected cow in Iowa and was provided by the Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brooking. All viruses were multiplied in Madin-Darby bovine kidney cells in minimum essential medium containing Earle's balanced salts (Gibco, Grand Island, N.Y.) plus 10% fetal bovine serum. Madin-Darby bovine kidney cells and fetal bovine serum were BVDV antibody free and BVDV virus free, as detected by indirect immunofluorescence assays (IFA) and virus neutralization (VN) assays.

Production of MAbs. MAbs D11 and B7 against BVDV field strain 87-2552 were produced by fusions of spleen cells from BALB/c mice immunized with virus preparations partially purified by sucrose gradient centrifugation and mouse myeloma P3x63Ag8.653 cells as described previously (21). MAbs D89, E15, and C17, produced against BVDV strain NADL (21), were also used in this study. MAb R54, produced against bovine herpesvirus 1, was used as a negative control. BVDV polyclonal antibodies developed against the Singer strain served as positive controls for this study. Subisotyping of MAbs was performed with a Mouse Typer subisotyping kit (Bio-Rad Laboratories, Hercules, Calif.).

ELISA. Nonidet P-40 extracts of Madin-Darby bovine kidney cells infected with BVDV strain 87-2552, NADL, or Singer were used to coat 96-well polystyrene plates. Enzyme-linked immunosorbent assays (ELISA) were performed by a standard procedure previously described (16). ELISA titers were expressed as the highest dilutions giving readings that were at least two times higher than that of the negative control.

IFA. The method for IFA was described previously (21). Briefly, BVDV-infected cells on 24-well plates were fixed with acetone and incubated with MAbs and subsequently with goat anti-mouse fluorescein isothiocyanate-labelled immunoglobulin G (IgG) (Hyclone, Logan, Utah). Cells were observed under a fluorescent microscope at a magnification of ×200. IFA titers of MAbs were expressed as the highest dilutions that showed bright fluorescent staining of virus-infected cells.

VN tests. VN tests were performed by plaque reduction assays, as described previously (21, 22). MAbs and BVDV polyclonal serum were tested for VN titers of each BVDV strain used, 87-2552, NADL, and Singer. These viruses were compared for antigenic differences in their neutralizing patterns with the MAbs selected.

Serial 10-fold dilutions of MAbs were used in performing the ELISA, IFA, and VN assays described above.

Immunoprecipitation and SDS-PAGE. The specificities of MAbs for viral proteins synthesized in culture cells were detected by immunoprecipitation. This procedure consisted of incubating virus-infected cell lysate and MAbs or BVDV polyclonal serum and subsequently adding protein A–Sepharose CL-4B as previously described (21). Immunoprecipitated samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% acrylamide gel. After being dried, the gel was exposed to X-Omat AR-S film (Eastman Kodak Co., Rochester, N.Y.), which was developed after 3 days.

RESULTS

Production and characterization of MAbs. Two MAbs (designated D11 and B7) developed against BVDV field strain 87-2552 strongly neutralized the virus, with a VN titer of $1:10^4$. The ELISA and IFA titers for these two MAbs were $1:10^4$ and $1:10^5$, respectively (Table 1). The subisotypes for these MAbs were determined to be IgG1 for D11 and IgG3 for B7. Immunoprecipitation revealed that both MAbs precipitated the viral protein gp48 (Fig. 1).

Comparison of BVDV field strain 87-2552 and vaccine

TABLE 1. Characterization of gp48-specific[a] MAbs for BVDV strain 87-2552

MAb	Titers			Subisotype[b]
	ELISA	VN	IFA	
D11	10,000	10,000	100,000	IgG1
B7	10,000	10,000	100,000	IgG3

[a] Specific proteins were determined by immunoprecipitation.
[b] Subisotypes of MAbs were determined with a Mouse Typer subisotyping kit (Bio-Rad).

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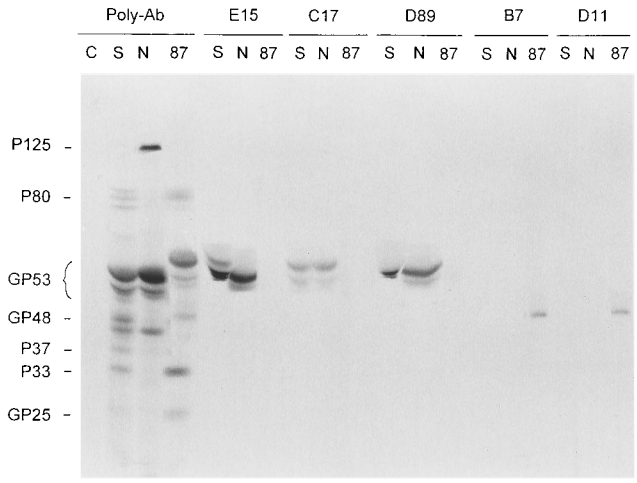

FIG. 1. Immunoprecipitation of BVDV 87-2552 (87), NADL (N), and Singer (S) polypeptides with different MAbs and polyclonal antibodies (Poly-Ab). Immunoprecipitated ^{35}S-labelled viral proteins were analyzed by SDS-PAGE and subsequently by autoradiography. The positions of selected proteins are indicated to the left of the gel. C, uninfected cell control.

strains NADL and Singer. The major purpose of our study was to detect antigenic differences between field strain 87-2552 and vaccine strains, NADL and Singer, of BVDV. Therefore, MAbs against strains 87-2552 and NADL were used to react with different virus strains in ELISA, IFA, VN tests, and immunoprecipitation assays. Table 2 presents the ELISA results of cross-reactions. Although MAbs D11 and B7 reacted with strains NADL and Singer, they demonstrated higher ELISA titers for their homologous virus strain 87-2552. The titer of MAb D11 for strain 87-2552 was 100-fold higher than its titer for strain Singer and about 1,000-fold higher than its titer for strain NADL. The titer of MAb B7 for virus 87-2552 was 100-fold higher than its titer for strain NADL and 10-fold higher than its titer for strain Singer. On the other hand, the titers of MAbs D89, E15, and C17 were 10- to 100-fold higher for strains NADL and Singer than their titers for strain 87-2552.

The IFA titers of these MAbs for different virus strains are shown in Table 3. The IFA titers of MAbs D11 and B7 were 1,000-fold higher for 87-2552-infected cells than they were for NADL- and Singer-infected cells. However, MAbs D89, C17, and E15 demonstrated 10-fold-higher titers for strains NADL and Singer than for strain 87-2552.

VN tests were performed by plaque reduction assays, and the results are presented in Table 4. MAbs D11 and B7 strongly neutralized strain 87-2552, reaching a VN titer of greater than 1:10^4. However, they did not neutralize strains NADL and Singer. MAbs D89, C17, and E15 neutralized both

TABLE 2. ELISA titers of MAbs for BVDV strains

Strain	Titer of MAb[a]					
	D11	B7	D89	C17	E15	R54
87-2552	10,000	10,000	1,000	1,000	1,000	10
Singer	100	1,000	100,000	10,000	100,000	10
NADL	10	100	100,000	10,000	100,000	10

[a] Data are averages of three values. MAbs D11 and B7 were raised against BVDV strain 87-2552; MAbs D89, C17, and E15 were raised against BVDV strain NADL, and MAb R54 against bovine herpesvirus 1 was used as a negative control.

TABLE 3. IFA titers of MAbs for BVDV strains

Strain	Titer of MAb[a]					
	D11	B7	D89	C17	E15	R54
87-2552	100,000	100,000	1,000	1,000	1,000	0
Singer	100	100	10,000	10,000	10,000	0
NADL	100	100	10,000	10,000	10,000	0

[a] See Table 2, footnote *a*.

NADL and Singer strains, with titers greater than 1:10^4, but did not neutralize strain 87-2552. The VN test appeared to be the best method for detecting the antigenic diversity of BVDV strains.

Immunoprecipitation was performed to detect the specificities of MAbs for BVDV proteins. Figure 1 shows that MAbs D11 and B7 were specific for gp48 of strain 87-2552 but did not precipitate the same protein from NADL- or Singer-infected cells. MAbs D89, C17, and E15 immunoprecipitated gp53 from NADL- and Singer-infected cells but not from 87-2552-infected cells. The results indicated that either strain 87-2552 lacks the epitopes for MAbs D89, C17, and E15 on gp53 or the affinities of these epitopes to these MAbs are very weak and the MAbs therefore cannot detect this protein. Apparently, gp48 is an important protein that is involved in VN and infection for strain 87-2552. Either strains NADL and Singer lack the epitopes for MAbs D11 and B7, or D11 and B7 do not have high affinities to the epitopes from NADL and Singer strains. Figure 1 shows that MAbs D11 and B7 recognized gp48 of strain 87-2552 (lanes 87) but did not react with any viral proteins of strains NADL (lanes N) and Singer (lanes S). Conversely, MAbs D89, C17, and E15 precipitated gp53 of strains Singer and NADL but did not recognize the same protein from strain 87-2552. Polyclonal BVDV antibodies precipitated BVDV-specific proteins p125, p80, gp53, gp48, p37, p33, and gp25 from BVDV strains, not from uninfected cell lysates. The gp53 and gp48 BVDV viral proteins were present in all three strains tested.

DISCUSSION

Extensive research has been carried out on antigenic variations in BVDV strains by using neutralizing MAbs (7–10, 17, 21). On the basis of reactivities with MAbs, BVDV strains have been segregated into groups (1, 14, 21). In our previous study (21), a panel of MAbs specific for gp53 of BVDV strain NADL was used to detect the antigenic diversities of 40 BVDV strains. Most interestingly, one cytopathic strain, 87-2552, was not neutralized by any MAb. In this study, two neutralizing MAbs, D11 and B7, were produced against strain 87-2552 and they neutralized only the field strain, not the vaccine strains NADL and Singer. In addition, MAbs against strain NADL did not neutralize this field strain. These results demonstrated that

TABLE 4. VN titers of MAbs for BVDV strains

Strain	Titer of MAb[a]					
	D11	B7	D89	C17	E15	R54
87-2552	10,000	10,000	<100	<100	<100	<100
Singer	<100	<100	10,000	10,000	10,000	<100
NADL	<100	<100	10,000	10,000	10,000	<100

[a] See Table 2, footnote *a*.

this field strain is antigenically diverse, compared with vaccine strains, in gp53 and gp48 epitopes.

Immunoprecipitation analysis showed that MAbs D11 and B7 were specific for BVDV protein gp48. BVDV has two major glycoproteins, gp53 and gp48, in the virus envelope (9). A 116-kDa glycoprotein is the precursor of gp53 and gp62, and the latter is subsequently cleaved to give gp48 and gp25 (6). gp53 is a very important viral protein that is involved in VN and infection (1, 9, 15, 21). There are very few reports of the function of gp48, but apparently gp48 is not as important as gp53 in VN (4, 21). Recently, Kwang et al. (13) reported that gp48 is also an important major glycoprotein for BVDV. In this study, MAbs specific for gp48 showed high VN titers, which shows the neutralizing epitopes on gp48. Field strain 87-2552 was not neutralized by our panel of gp53-specific MAbs, and immunoprecipitation did not precipitate gp53 from this field strain, suggesting that gp53 is not dominant in this field strain and that gp48 is more important for this strain in VN and infection. Using a panel of gp48-specific MAbs, Boulanger et al. (4) showed low VN titers of gp48-specific MAbs for BVDV strains, and titers were enhanced by goat anti-mouse IgG serum. Therefore, they suggested that these MAbs are not directed against critical VN sites. Three MAbs specific for gp42 of hepatitis C virus, equivalent to gp48 of BVDV, with low VN titers were also produced (19). Our previous study described a neutralizing gp48-specific MAb that did not neutralize the majority of BVDV strains. The function of gp48 of BVDV still needs to be studied.

MAbs against this field strain detected NADL and Singer strains in ELISA and IFA, but not in VN and immunoprecipitation tests. The same thing happened when MAbs against strain NADL were used to test the field strain. The explanation for this may be that MAb-binding epitopes occur on heterologous viruses and can react with heterologous MAbs in ELISA and IFA. However, these are not neutralizing epitopes; thus, they are not involved in VN. In immunoprecipitation assays, heterologous MAbs and viral proteins cannot bind to each other, possibly because these virus strains are so different in antigenicity and the affinities of these MAbs and viral proteins are very weak. This study also showed that the VN test is the best assay for distinguishing between virus strains with MAbs.

Our results address the importance of BVDV antigenic diversity in cattle. Conventional vaccines that are effective for protecting against either homologous strains or strains with only slight antigenic variations have been produced against prototype strains. If a herd is exposed to a new BVDV strain with different antigenicity (3), such as field strain 87-2552, cattle may not be fully protected. Therefore, more efficacious and safe vaccines are needed to prevent BVDV infection (2, 3).

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