Monoclonal Antibodies That Distinguish between Encephalitogenic Bovine Herpesvirus Type 1.3 and Respiratory Bovine Herpesvirus Type 1.1

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Seven mouse hybridoma cell lines producing monoclonal antibodies (MAb) against an encephalitogenic strain of bovine herpesvirus type 1.3 (BHV-1.3) were established. The clones producing MAb were selected to be specific for BHV-1.3 by enzyme-linked immunosorbent assay. Only 11B neutralized virus without complement. With the addition of complement, five of the MAb neutralized BHV-1.3 but not the respiratory strain BHV-1.1. The anti-BHV-1.3-specific MAb Q10B, L6G, and L11B precipitated glycoproteins from BHV-1.3 that were analogous to the gl, gII, and gIV glycoproteins of BHV-1.1, respectively. The other four MAb precipitated unknown proteins. None of the anti-BHV-1.3 MAb precipitated BHV-1.1 glycoproteins. The majority of the anti-BHV-1.3 MAb did not react with BHV-1.1 by immunoblotting, but OTE (unknown protein pattern by radioimmunoprecipitation) was reactive with five proteins (M_s of 33,000, 43,000, 70,000, 141,000, and 190,000) of BHV-1.3 and with a different pattern of proteins of BHV-1.1 (M_s of 30,000, 38,000, 83,000, and 144,000). Two of the MAb, L6G and OTE, conjugated with peroxidase were found to be useful for detecting BHV-1.3 antigen by immunochrometry in Formalin-fixed brain tissue from experimentally infected calves.

Armstrong et al. (3) identified the virus isolated by Madin et al. (28) from cattle afflicted with respiratory disease (infectious bovine rhinotracheitis [IBR]) in California (37) and Colorado (32) as a herpesvirus. Herpesviruses have since been isolated from a variety of other diseases in cattle: infectious pustular vulvovaginitis (IPV) (23), conjunctivitis or keratoconjunctivitis (1, 26, 35), aborted bovine fetuses (30), meningoencephalitis (19), mastitis (36), and enteritis (24). As reviewed by George (21), the encephalitic form of bovine herpesvirus (BHV) infection has been reported to occur in calves in the United States, Europe, South America, Canada, and Australia.

Initially, conventional serological means were not successful in distinguishing among isolates of BHV (7, 23). As a classification scheme evolved, the viruses that were serologically similar to IBR virus and IPV virus became designated BHV type 1 (BHV-1) instead of IBR virus (22). Three subtypes of BHV-1 have been distinguished by restriction endonuclease analysis (16, 17, 31), reactivity of monoclonal antibodies (MAb) (20), and protein patterns after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5, 38). The respiratory isolates associated with IBR were classified as BHV-1.1; genital isolates associated with IPV were classified as BHV-1.2a and -1.2b; and neurological disease (encephalitogenic) isolates were classified as BHV-1.3a and -1.3b (31). At about the same time, Australian researchers designated the encephalitogenic strain bovine encephalitis herpesvirus because the differences noted in restriction endonuclease DNA fingerprints between encephalitis and respiratory-genital isolates were much greater than those noted between respiratory and genital isolates (8, 10, 42). The DNA restriction endonuclease fragmentation patterns of two BHV-1.3 isolates from Texas differed completely from those of BHV-1.1 isolates, but they were similar to those of the N-569 prototype encephalitic (BHV-1.3) virus from Australia (19) and the A-663 isolate from Argentina (11).

In early investigations of the disease, it was noted that occasionally young cattle infected with BHV developed nonparulent leptomenigitis and encephalitis (27). Neurological signs included muscular incoordination, aimless circling, recumbency, blindness, convulsions, and death (11, 18, 19, 34). The histopathology of such cases revealed perivascular cuffs with mononuclear cells present throughout the brain, especially in the cerebrum (6).

The encephalitogenic strains were found to be different from the strains of IBR virus causing IPV when neutralization kinetics were studied with hyperimmune rabbit antiserum (5). In a recent study, serum cross-neutralization tests again failed to reveal differences among several isolates representative of BHV-1, but kinetic neutralization tests showed a significant difference between an encephalitogenic strain of BHV-1.3 and the BHV-1.1 types tested (9).

Identification of important epitopes on viral glycoproteins is a key step in the construction of synthetic peptide or recombinant DNA vaccines or in selection of MAb that may be useful as diagnostic reagents. Individual epitopes on the BHV-1 glycoproteins have been identified with specific mouse MAb (14, 33, 44, 45). Collins et al. (13) and Ayers et al. (4) found that the recognition of BHV-1 glycoproteins by mouse MAb and the bovine immune response were different when compared in a competition enzyme-linked immunosorbent assay (ELISA) and radioimmunoprecipitation (RIP) tests. The purpose of the present study was to prepare and select mouse MAb to BHV-1.3 glycoproteins that could be used to distinguish isolates of this type from the BHV-1.1 type.
MATERIALS AND METHODS

Animals. BALB/c mice (Charles River) were used as donors of spleen cells, thymocytes, peritoneal macrophages, and ascites for the hybridoma technique. Newborn colostrum-deprived Holstein calves were obtained from local producers.

Viruses. BHV-1.1 (strain CSU 10902-82) was isolated from an outbreak of infectious bovine rhinotracheitis in Colorado and is identical to the Cooper strain. An encephalitogenic strain of BHV-1.3 (strain 30326) was isolated from brain tissue of calves with encephalitis in Texas (18). Herpes simplex virus type 1 (HSV-1) was from the American Type Culture Collection (Rockville, Md.; strain no. ATCC VR-733).

Virus propagation and purification. Madin-Darby bovine kidney (MDBK) cells grown in minimal essential medium (MEM) with 5% fetal bovine serum (FBS), 10 µg of gentamicin per ml, 290 µg of t-glutamine per ml, and 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer were infected at a multiplicity of one 50% tissue culture infective dose (TCID50) of BHV-1 per cell. Most cells showed cytopathic effects about 36 to 48 h postinfection, and virus was purified from these cultures as described previously (14). Fractions containing protein, as determined with bicinchoninic acid (41), and viral antigen, as detected by ELISA, were pooled, diluted with TEN buffer (6.055 g of Trizma base, 0.372 g of EDTA disodium salt, and 8.77 g of NaCl per liter, adjusted to pH 7.4), and then pelleted and resuspended in TEN buffer before being used as purified viral antigens.

Production of anti-BHV-1.3 MAb. Purified BHV-1.3 (100 µg of protein) was mixed vigorously with an equal volume of Ribi adjuvant (RIBI Immunocoh Research, Inc., Hamilton, Mont.) and injected subcutaneously three times at 2-week intervals into 8- to 12-week-old female BALB/c mice. The final injection of 100 µg of purified virus in TEN buffer without adjuvant was given intraperitoneally 2 weeks after the last subcutaneous injection. BALB/c mouse spleen cells collected 3 days after the last immunization were fused with SP2/0 AG-14 myeloma cells at a ratio of 4:1 in 1 ml of 50% polyethylene glycol (PEG 4000; E. Merck, Darmstadt, Germany)–10% dimethyl sulfoxide in RPMI 1640 HM medium (Sigma Chemical Co., St. Louis, Mo.) for 2.5 min. The fused cells were diluted, pelleted, and resuspended in 35 ml of complete medium with 15% FBS and 5 µg of a B-cell mitogen from Salmonella typhimurium (RIBI Immunocoh Research) per ml (15). The cells were dispensed (105 cells per well) into 96-well flat-bottomed Microtest III tissue culture plates (Becton Dickinson, Lincoln Park, N.J.) with thymocyte feeder cells. On each of the following 3 days, 100 µl of HAT medium (RPMI 1640 complete medium containing 1 × 10–8 M hypoxanthine, 4 × 10–7 M aminopterin, and 1.6 × 10–5 thymidine [Sigma Chemical Co.] with 10% FBS) was added to each well. Ten to 14 days after fusion, the hybridomas were screened for antibodies against BHV-1.3 by ELISA. Ascitic fluids were raised in BALB/c mice primed with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Sigma Chemical Co.) 2 to 4 weeks before intraperitoneal inoculation of 5 × 106 selected hybridoma cells that had been cloned three times by limiting dilution.

Purification of MAb. Antibodies in clarified (200 × g) ascitic fluids were salted out with 45% ammonium sulfate. The protein content and specific antibody activity of the dialyzed fraction were determined with bicinchoninic acid and by ELISA, respectively. Partially purified antibodies were aliquoted and stored at −20°C.

Determination of MAb isotype. An ELISA isotyping kit, Screen Type (Boehringer Mannheim, Indianapolis, Ind.), was used to determine the class and subclass of each MAb.

Other MAb. The following BHV-1-specific MAb were obtained from previous studies: D9 (g1 specific) and F2 (gIII specific) (14); 5306 (g1 specific) and 1102 (gIV specific) (29); and 9D6 (gV specific) (25).

ELISAs for detection of specific antibodies to BHV-1.3. Antigens were air-dried at 37°C overnight onto flat-bottomed 96-well ELISA plates (Immulon 2; Dynatech Laboratories Inc., Alexandria, Va.). Three adjacent columns were coated with BHV-1.1 antigen, BHV-1.3 antigen, or uninfected MDBK cell lysates in sodium borate buffer (0.01 M, pH 9.0). The optimal concentrations of antigens per well were 300 ng of purified virus, a 1:80 dilution of infected MDBK lysate, and a 1:160 dilution of uninfected cell lysate. Each well was blocked at 37°C for 1 h with 200 µl of Blotto (2) containing 2% skim milk. After the blocking solution was removed, 50 µl of a culture supernatant was added to each of the three test wells for 1 h at 37°C. After the wells were rinsed five times with PBS-Tween buffer (1.8 mM NaH2PO4·H2O, 8.4 mM Na2HPO4, 150 mM NaCl, 0.05% Tween 20 [pH 7.4]), 50 µl of biotinylated rabbit anti-mouse immunoglobulin G (IgG; heavy and light chains; Zymed Laboratories Inc., South San Francisco, Calif.) per well was incubated at 37°C for 0.5 h. After rinsing, 50 µl of strepavidin-peroxidase conjugate (Zymed Laboratories Inc.) was added for 0.5 h at 37°C. After rinsing, 100 µl of the chromogen 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co.) with 0.0045% hydrogen peroxide was added for 10 min at room temperature in the dark. The reaction was stopped by adding 50 µl of 1 N H2SO4. A supernatant was presumed to contain antiviral antibodies if the optical density at 450 nm of the well with viral antigen but not the well with uninfected MDBK lysate was at least threefold higher than that of a negative control well coated with 10% FBS. Four different antigens, BHV-1.3, BHV-1.1, herpes simplex virus type 1, and uninfected MDBK cell lysates, were subsequently used to select specific antibodies against BHV-1.3.

Immunoblotting. All immunoblotting procedures were conducted at room temperature. Gels with viral proteins separated by PAGE were equilibrated in electrophoresis transfer buffer (0.025 M Tris, 0.192 M glycine, 20% [vol/vol] methanol). After transfer to a nitrocellulose membrane (Transblotter; Bio-Rad Laboratories, Richmond, Calif.) at 11 V overnight, the membrane was rinsed with distilled water and blocked with 10% skim milk in PBS (pH 7.4) for 1 h. Blocked blots were rinsed with washing buffer (PBS with 500 mM NaCl, 1 mM EDTA, and 0.5% Tween 20) and dried. Strips cut from the blots were immersed in the MAb to be tested and incubated for 1 h. After the strip was rinsed three times with washing buffer, bound antibody was detected by immersion in alkaline phosphatase-conjugated goat anti-mouse IgG (heavy and light chains; Zymed Laboratories Inc.) at a dilution of 1:250 in washing buffer containing 10% skim milk. After 1 h, the blot was washed again and rinsed with distilled water before addition of chromogen (nitroblue tetrazolium) and substrate (5-bromo-4-chloro-3-indolyl phosphate) for 30 min. The enzyme reaction was stopped by rinsing with distilled water.

Immunocytochemistry. Cultures of MDBK cells were grown in MEM with 10% FBS in 24-well tissue culture plates seeded with 3 × 105 cells per well. On the next day the monolayers were rinsed three times with PBS and infected with BHV-1.1 or BHV-1.3 at a multiplicity of infection of 1.0. At 0 h postinfection, the plates were rinsed with PBS and dried at room temperature. After fixation with acetone at 4°C for 10 min or with 1% Formalin for 24 h at room temperature, the
plates were blocked with 10% normal horse serum in PBS for 30 min and then washed three times with PBS. Anti-BHV-1.3 MAb were added for 1 h at 37°C. After three washes, biotinylated rabbit anti-mouse Ig (Zymed Laboratories Inc.) was added for 1 h at 37°C. The plates were again washed before incubation with strepavidin-peroxidase conjugate (Zymed Laboratories Inc.) for 30 min at 37°C. The plates were washed five times before the addition of 3,3'-diaminobenzidine and H2O2. After 15 min, the plates were washed with PBS and then observed microscopically.

Immunocytochemistry was also performed on brain sections obtained from BHV-1.3-infected calves. Colostrum-deprived newborn calves were given 105 TCID50 of virus by intranasal inoculation and killed 9 to 10 days later, when signs of encephalitis developed. Sections of brain were fixed in 10% buffered Formalin. Various dilutions of purified ascitic fluids containing the anti-BHV-1.3 MAb were screened for reactivity by detection with the avidin-biotin-peroxidase complex method, as described previously (40).

**Virus neutralization by plaque assay.** Twofold dilutions of MAb were mixed with a dilution of virus sufficient to give a countable number of plaques in the presence or absence of 5% guinea pig serum (Organon Teknika, West Chester, Pa.). This mixture was incubated for 18 h at 37°C and then inoculated into duplicate wells of monolayered bovine turbinate cells, 0.2 ml per well, in six-well tissue culture plates. These plates were rocked on a rocking platform at 37°C for 90 min, washed, and overlaid with MEM with 0.6% agarose and 1% fetal bovine serum. After 3 days at 37°C, the overlays were removed, the monolayers were stained with 1% crystal violet in 20% ethanol, and plaques were counted. The titer was recorded as the highest dilution of antibody that reduced the number of plaques by at least 50% compared with controls without antibody.

**Radiolabeling.** Labeling with [35S]methionine and [3H]glucosamine was performed as described previously (14), with primary cultures of bovine embryonic lung. The 35S radioactive label consisted of [35S]methionine and [35S]lysine (Trans-3S-S-Label, 1,100 Ci/mmol; ICN Biomedicals Inc., Costa Mesa, Calif.) and was added (100 µCi/ml) in methionine-free medium to infected cell cultures between 6 and 20 h after virus inoculation. [1-3H]glucosamine (70 Ci/mmol; DuPont-NEN, Boston, Mass.) was added to infected cell cultures during the same period. For a negative control, ascites induced by SP2/0 cells was used in the assay.

**RIP and SDS-PAGE.** The radioimmunoprecipitation (RIP) and SDS-PAGE assays were carried out as described previously (14). The secondary immunosorbent was either rabbit anti-mouse IgG immunobeads (Bio-Rad Laboratories Inc.) or protein A-Sepharose beads (Sigma Chemical Co.).

**Conjugation of anti-BHV-1.3 MAb with horseradish peroxidase.** The conjugation of horseradish peroxidase to antibody was a periodate-mediated procedure (43).

**RESULTS**

**Production and characteristics of MAb to BHV-1.3.** The average fusion efficiency for four different fusions was 91% (3,232 of 3,552 wells). The initial screening ELISA for hybridomas against purified BHV-1.3 yielded 36% (1,172 of 3,232 wells) positives. In a second screening ELISA for specificity, the initially positive hybridomas were tested for reactivity to purified BHV-1.1 and herpes simplex virus type 1. After the specificity screening, 9% (102 of 1,172) of the hybridomas were selected as being specific for BHV-1.3. Seven stable and specific anti-BHV-1.3 antibody-producing monoclonal hybridomas were selected for later experiments after several passages and dilution clonings. Ascitic fluids for each cloned hybridoma were salted out with 43% ammonium sulfate and dialyzed against TEN buffer. The isotype of three antibodies, L1B, P12E, and C7C, was IgG1; that of O7E and H11F was IgG2b; Q10B was IgG3; and L6G was IgM (Table 1). All the antibodies had kappa light chains.

The specificities of the anti-BHV-1.3 MAb were also tested after conjugation with horseradish peroxidase. The conjugated anti-BHV-1.3 MAb did not bind to BHV-1.1, nor did adsorption of anti-BHV-1.3 MAb conjugates with purified BHV-1.1 reduce the reactivity of these conjugates with BHV-1.3 by ELISA. Additionally, different proteins such as horse serum, rabbit serum, normal mouse serum, antibodies specific for other viruses and bacteria, SP2/0 ascites, ovalbumin, and bacterial glycolipids did not compete with MAb-horseradish peroxidase conjugates for BHV-1.3.

**Virus neutralization.** The plaque reduction titers for seven of the BHV-1.3-specific MAb detected by ELISA are shown in Table 1. Only L1B neutralized virus without complement. With the addition of complement, five of the MAb neutralized virus, the most efficient being L1B, followed by H11F and Q10B.

**RIP and SDS-PAGE.** Viral proteins from lysates of [35S]methionine-labeled infected cells were precipitated by MAb and analyzed by SDS-PAGE under reducing conditions. The immunoprecipitation patterns are compared with those of BHV-1.1 proteins by BHV-1.1-specific MAb in Fig. 1. The anti-BHV-1.3-specific MAb Q10B, L6G, and L1B precipitated proteins from BHV-1.3 recognized by H11F in the far right lane.

![FIG. 1. RIP and SDS-PAGE of [35S]methionine-labeled viral proteins from BHV-1.1 (R, respiratory isolate) and BHV-1.3 (E, encephalitogenic isolate). MAb (lanes from left to right): D9 (gV specific), Q10B and F2 (gH specific), L6G and 1102 (gV specific), and L1B. Unknown proteins from BHV-1.3 were recognized by H11F in the far right lane.](http://cvi.asm.org/)
glycoproteins analogous to gIL (130,000 M, [130K], 68K, and 53K cleavage products), gIII (91K), and gIV (71K) of BHV-1.1, respectively. The same RIP patterns were found with [3H]glucosamine-labeled viral proteins (data not shown). The precipitated glycoproteins from BHV-1.3 were also identified by RIP with MAb prepared against BHV-1.1 that cross-reacted with gI, gII, and gIV from both viruses (12). None of the anti-BHV-1.3 MAb precipitated BHV-1.1 glycoproteins. The immunoprecipitation pattern of protein bands with H11F was unique to BHV-1.3 (major band at 100K); an analogous protein from BHV-1.1 was not identified. The anti-BHV-1.3 MAb O7E also precipitated unidentified viral proteins, while C7C and P12E did not precipitate viral proteins.

Reactivity of anti-BHV-1.3 MAB to BHV-1.3 and BHV-1.1 by immunoblotting. The BHV-1.3-specific MAB were further characterized by immunoblotting. Proteins from BHV-1.1 and BHV-1.3 were separated under denaturing and reducing conditions by PAGE and then electrophoretically transferred to a nitrocellulose membrane. The reactivities of anti-BHV-1.3 MAb and anti-BHV-1.1 MAb are compared in Fig. 2. The majority of the anti-BHV-1.3 MAb did not react with BHV-1.1. A very faint band at 71K in BHV-1.3 was recognized by MAB L1B (gIV by RIP); L6G (gIII by RIP) was reactive with 40K, 51K, 69K, and 91K proteins; Q10B (gI by RIP) was reactive with a 74K protein; O7E (unknown protein pattern by RIP) was reactive with five proteins of 33K, 43K, 70K, 141K, and 190K, of BHV-1.3 and with a different pattern of BHV-1.1 proteins at 30K, 38K, 83K, and 144K; P12E bound prominently to a 45K protein and faintly to 33K, 72K, and 146K proteins. The anti-BHV-1.1 MAb 5306 (gI specific) reacted similarly with the gI precursor protein of BHV-1.1 and BHV-1.3. Anti-BHV-1.1 MAB 1102 and 9D6 (gIV specific) reacted specifically with BHV-1.1.

Immunocytochemistry. The anti-BHV-1.3 MAb were screened for reactivity on monolayers of MBDK cells that had been infected with BHV-1.3. All the anti-BHV-1.3 MAb stained BHV-1.3 antigen but not BHV-1.1 antigen in the acetone-fixed monolayers. None of the MAb stained the viral antigen from the Formalin-fixed monolayers. Infected cells surrounding developing plaques in monolayers of MBDK cells were clearly seen by immunocytochemistry (Fig. 3). Staining was present in all parts of the infected cells.

The seven MAb reactive with BHV-1.3, two were found to be useful for detecting viral antigen in Formalin-fixed infected brain tissue. Staining with either MAb L6G or O7E demonstrated the presence of viral antigen in infected neurons in brain sections (Fig. 4). Of the two MAb, O7E gave the stronger signal and seemed to react with a nuclear antigen (Fig. 4A). MAb L6G reacted with neurons that were degenerating, with most of the stain present throughout the infected cells (Fig. 4B). This was similar to the pattern seen with the cross-reactive anti-BHV-1.1 gI MAb D9, which also detected BHV-1.3 viral antigen in brain sections (data not shown).

**DISCUSSION**

By endonuclease restriction maps and protein patterns, BHV-1 can be divided into three major subtypes (31). Although the three subtypes are antigenically and genetically related, BHV-1.3 shows different biological behavior than the other two subtypes. Mouse MAb specific for BHV-1.3 were produced and used to characterize the differences between BHV-1.1 and BHV-1.3. ELISA was used for initial screening of the antibody-producing hybridomas. Ninety percent of the MAb against BHV-1.3 were also reactive with BHV-1.1; however, a second screening ELISA allowed selection of anti-BHV-1.3-specific MAb and indicated similarities but also antigenic differences between the two viruses.

Neutralization was found mainly with the MAb that reacted with the viral glycoproteins. MAb Q10B, specific for gI, neutralized virus in the presence of complement, similar to previous findings with MAb to gIL of BHV-1.1 (14, 44). MAb L1B, specific for glycoprotein gIV, showed the highest titers with and without complement present, and this is also similar to previous results found with neutralizing MAb to BHV-1.1 (29, 39, 44). The BHV-1.3 glycoprotein recognized by the neutralizing MAb H11F could not be directly compared with an equivalent protein of BHV-1.1. However, the molecular mass (approximately 100 kDa) is similar to that of glycoprotein gIL, a minor glycoprotein on BHV-1.1 (44), and the neutralization activity of this MAb may be the result of recognition of a similar viral surface glycoprotein. Neutralization kinetics
were shown to be different between BHV-1.1 and BHV-1.3 with polyclonal antiserum, but cross-neutralization tests failed to reveal differences between respiratory and meningoencephalitic isolates of BHV (9). Bratanich et al. (9) concluded that cross-reactivity between the strains of BHV used in current vaccines will probably protect against the BHV-1.3 group.

In immunoprecipitation tests, two kinds of immunoadsorbents (rabbit anti-mouse IgG immunobeads and protein A-Sepharose beads) were used to adsorb the antigen-antibody immune complexes. Certain immune complexes cannot be adsorbed by one immunoadsorbent or another; therefore, two immunoadsorbents were mixed for use in RIP assays. Five MAb (L1B, L6G, Q10B, H11F, and 07E) precipitated BHV-1.3 proteins. None of the anti-BHV-1.3 MAb precipitated BHV-1.1 in the RIP assay.

Immunoblotting was performed for further characterization of the anti-BHV-1.3 MAb. Five of the seven stable MAb (L1B, L6G, Q10B, P12E, and 07E) reacted with BHV-1.3 proteins by immunoblotting. Only MAb 07E reacted with BHV-1.1 in an immunoblot, and it gave a different pattern than it did against BHV-1.3. Several MAb reacted with more than one viral protein in RIP and immunoblot assays. This observation was consistent with other reports (14, 20, 33, 45). The multiple bands may be due to the reaction of MAb with precursors or cleavage products of the same viral protein or to a common epitope on the different viral proteins (45). By calculating the molecular weights of the proteins precipitated and comparing the immunoprecipitation patterns with those of the anti-BHV-1.1 MAb (specific for gI, gIII, and gIV), MAb Q10B was specific for BHV-1.3 gI (74K, cleavage product of 135K); L6G recognized gIII (91K); and L1B was specific for gIV (71K).

Minor differences were noted in the molecular weights of the corresponding glycoproteins between BHV-1.1 and BHV-1.3. Additional lower-molecular-weight protein bands appeared with the immunoprecipitation of gIII from BHV-1.3. These bands were also present in BHV-1.1 but were not recognized with MAb F2 (12). A detailed study of the epitopes and biochemical properties of the BHV-1.3 glycoproteins remains to be carried out.

Two MAb, L6G and 07E, conjugated with peroxidase were useful for detecting BHV-1.3 viral antigen in infected cell cultures or in brain tissue that had been fixed with 10% Formalin. The staining patterns were somewhat different; 07E stained nuclei in degenerating neurons, while L6G stained entire cells, particularly those in advanced degeneration. One or both of these MAb may be useful in correlating histopathologic changes in animals with BHV-1.3 encephalitis.

The subtypes of BHV-1 are closely related, as evidenced by the large numbers of MAb that had to be screened in order to select a few that were specific for BHV-1.3. Two MAb specific for BHV-1.3 identified BHV-1.3 expression in Formalin-fixed brain tissue. We expect that they will be able to identify infection by this virus in other tissues and perhaps reveal a unique tropism for this subtype of BHV-1. Some of the MAb might be used in studies designed to detect antigenic differences that can be related to tropism differences among the subtypes of viruses in this group. The serological tests that are currently available do not reveal the prevalence of BHV-1.3 infections in cattle. Some of the MAb described here now offer the possibility for a diagnostic test specific for detection of BHV-1.3 infection.

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