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Identification of a Neutralizing Epitope Shared by Bluetongue Virus Serotypes 2 and 13

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The serotypes of bluetongue virus (BTV) are classified by differences in neutralization commonly induced by P2, a major surface protein. A BTV serotype 13 (BTV-13) monoclonal antibody, 4B13-207A, immunoprecipitated P2s of BTV-13 and BTV-2 and also neutralized both viruses. These data indicate that P2s from BTV-13 and BTV-2 share a common neutralizing epitope that is not detected by neutralizing polyclonal antibody to BTV-13.

Bluetongue virus (BTV) is an arthropod-transmitted orbivirus of the family *Reoviridae* which infects cattle, sheep, and wild ruminants. It is of great economic importance because the export of BTV-infected animals and germ plasm is restricted in world markets (16). More than 23 serotypes of the virus are recognized worldwide, and BTV serotypes 2 (BTV-2), 10, 11, 13, and 17 have been found within the United States. The virus is composed of seven polypeptides and 10 segments of double-stranded RNA. Five of the seven polypeptides (P1, P3, P4, P6, and P7) are found in the inner core of the virus, while the other two (P2 and P5) are found in the diffuse outer capsid (8, 23). Polypeptide P2 of the outer capsid confers serotype specificity and induces the production of neutralizing antibody and protective immunity (6, 8, 9). Accurate identification of serotypes is essential in the search for effective vaccines, especially since several serotypes may be present in an infected population and the virus has been proven capable of forming reassortants in culture (9) and in cattle (21), sheep (19), and the vector (18). Similarities and differences in the various serotypes of BTV and closely related viruses (e.g., epizootic hemorrhagic disease and Ibaraki viruses) are also important for monitoring disease epizootiology and tracing the evolutionary lineage of BTV (2, 3, 17).

Reference serotypes 2, 10, 11, 13, and 17 were obtained from the Arthropod-Borne Animal Diseases Laboratory, Agricultural Research Service, Denver, Colo. Cloned viruses were propagated in BHK or Vero cells as previously described (1). Briefly, virus propagated in BHK cells was extracted by sonication in 2 mM Tris hydrochloride buffer with 0.5% (vol/vol) Triton X-100. Virus released from the supernatant was purified by pelleting through a 40% (wt/vol) sucrose cushion at 76,000 × *g* for 90 min. To produce immune spleen cells for fusion, BALB/c or Robertsonian (RB/DF) mice were immunized subcutaneously with BTV-13 prepared by four serial passages in neonatal mice and emulsified in Freund adjuvant (1). Hybridomas were produced by the fusion of immunized spleen cells with P3X63.AG8.653 (10) or the Fox/NY myeloma (22) lines by the method outlined by Fazekas de St. Groth and Scheidegger (4). The fused cells from one spleen were plated in 10 microdilution plates on a feeder layer of mouse thymocytes. Hybridomas were selected which produced antibody that neutralized BTV-13 at 10 to 100 50% tissue culture infective doses and immunoprecipitated the serotype-specific polypeptide (P2) of BTV-13. These monoclonal antibodies (MAbs) were screened for specificity by testing for their neutralizing and immunoprecipitating activities against BTV-2, BTV-10, BTV-11, and BTV-17. The antibody isotypes were determined with a commercially available kit. Polyclonal antiserum to BTV-13 used in this study was obtained from mice immunized for the generation of MAb to BTV-13.

Samples were prepared for immunoprecipitation and electrophoresis by the method of Kessler (11, 12), using fixed *Staphylococcus aureus*. All antigens and antibodies were centrifuged at 134,000 × *g* for 30 min to reduce background, and saturating quantities were used in each step of the immunoprecipitation procedure. The bacteria were washed three times in immunoprecipitation buffer (0.1% sodium dodecyl sulfate, 0.5% Nonidet P-40, 0.2% deoxycholate, 10 mM Tris, 0.15 M sodium chloride [pH 8]). The following reagents were added sequentially to 100-μl portions of a 10% suspension of bacteria: (i) 30 μl of rabbit anti-mouse immunoglobulin (previously absorbed with an equal packed volume of cells of the type in which the radioactive BTV antigen preparation was produced); (ii) 30 to 300 μl of mouse serum of MAb; (iii) [^{35}S]methionine-labeled antigen (a lysate of BTV-infected BHK cells) prepared as described by Appleton and Letchworth (1). Samples were incubated on ice for 2 h and washed three times in immunoprecipitation buffer. Sodium dodecyl sulfate-gel electrophoresis was performed with 5% stacking gels and 7.5 or 10% separating gels by the method of Laemmli (14). Serum neutralization titers were expressed as the reciprocal of the dilution of serum or MAb that gave complete protection against the cytopathic effect of 10 to 100 50% tissue culture infectious doses of BTV in monolayers of Vero cells in 96-well microdilution plates.

2B13-268D, an immunoglobulin G1 MAb produced by fusion with the Fox/NY cell line and used as a control, neutralized only BTV-13 and none of the other serotypes of BTV (Table 1). Control mouse polyclonal antiserum to BTV-13 neutralized only BTV-13. In contrast, 4B13-207A, an immunoglobulin M MAb produced by fusion with P3X63.AG8.653, neutralized both BTV-13 and BTV-2. Both MAbs precipitated an antigen from BTV-13 which is deduced to be P2 (Fig. 1, lanes 4 and 5). In addition to precipitating P2 of BTV-13 (Fig. 2, lane 2), 4B13-207A

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TABLE 1. Neutralization of BTV serotypes

MAb or antiserum	Result for BTV serotype:				
	2	10	11	13	17
4B13-207A	+[a]	–	–	+[a]	–
2B13-268D	–	–	–	+[b]	–
BTV-13 antiserum	–	–	–	+[c]	–

[a] Ascites diluted to greater than 1/20,480 protected the monolayer of Vero cells.
[b] Ascites diluted to 1/640 protected the monolayer of Vero cells.
[c] Serum diluted to 1/320 protected the monolayer of Vero cells.

precipitated a 103,000-molecular-weight antigen from a BTV-2 extract (Fig. 2, lane 3). As expected, anti-BTV-13 serum obtained from mice used for the generation of anti-BTV MAbs precipitated a number of the BTV-13 proteins, including P2 (Fig. 3, lane 1). However, the same antiserum failed to precipitate P2 of BTV-2 (Fig. 3, lane 4) or of BTV-10 (Fig. 3, lane 8), although it precipitated all the other proteins, as it did in BTV-13. Neither 2B13-268D, a control MAb to BTV-13, nor 4B13-207A precipitated antigens from BTV-10 (Fig. 3, lanes 10 and 11). Negative results were also obtained for BTV-11, BTV-17, and mock-infected BHK cells (data not shown).

Thus, we have discovered a neutralizing epitope on P2 shared by BTV-13 and BTV-2. This result was unexpected, since to date most studies have shown no relatedness of P2 polypeptides from any of the BTV serotypes (5, 9, 13, 15, 20). No cross-hybridization of P2 RNAs (RNA-RNA) from the five domestic serotypes was seen in two recent studies performed under stringent conditions (5, 13). Peptide mapping experiments recently performed with staphylococcal protease V8 showed conservation of P5 but not P2 polypeptide sequences in domestic serotypes (15). Most importantly,

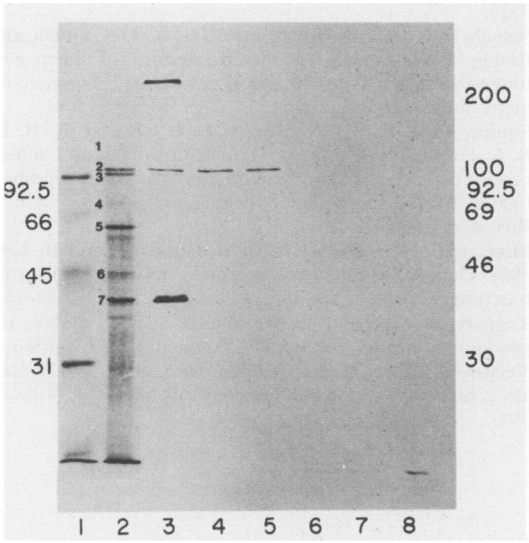

FIG. 1. Coelectrophoresis of nonradioactive whole BTV-13 proteins with immunoprecipitates of [^{35}S]methionine-labeled BTV-13 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lanes: 1, nonradioactive molecular weight protein standards; 2, BTV-13, with numbers identifying the constituent polypeptides according to Huismans et al. (8) (lanes 1 and 2 were stained with Coomassie blue); 3, anti-BTV-13 mouse serum; 4, MAb 4B13-207A; 5, control MAb 2B13-268D; 6, non-immune mouse serum control; 7, rabbit anti-mouse serum control; 8, ^{14}C-labeled molecular weight standards (lanes 3 through 8 are an autoradiograph of the dried gel).

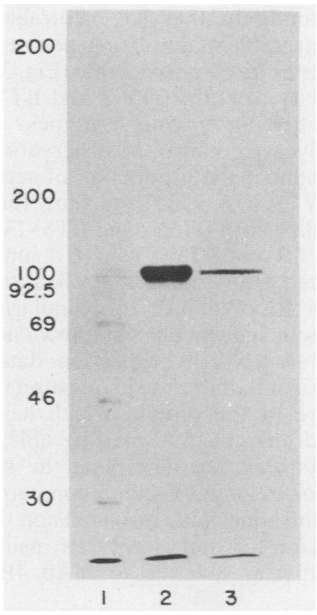

FIG. 2. MAb 4B13-207A precipitated only P2 polypeptides of both [^{35}S]methionine-labeled BTV-13 (lane 2) and [^{35}S]methionine-labeled BTV-2 (lane 3) polypeptides. Lane 1, ^{14}C-labeled molecular weight standards.

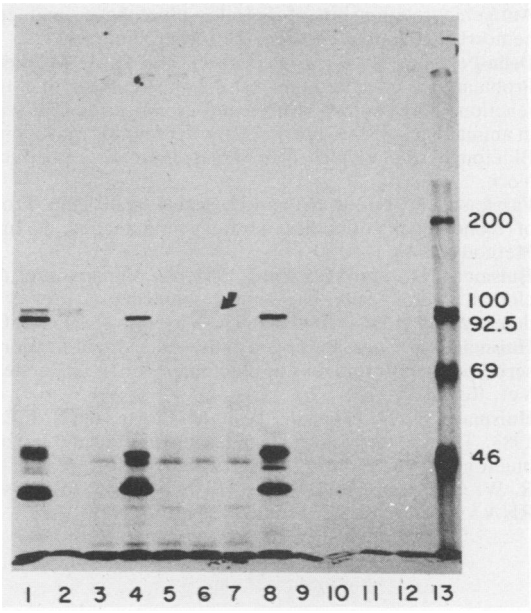

FIG. 3. Specificity of MAb 4B13-207A. In lanes 1 and 2, the antigen is [^{35}S]methionine-labeled BTV-13 antigen. Lanes: 1, BALB/c anti-BTV 13; 2, 2B13-268D. In lanes 3 through 7, the antigen is [^{35}S]methionine-labeled BTV-2. Lanes: 3, non-immune BALB/c serum; 4, BALB/c anti-BTV-13; 5, 2B13-268D; 6, 4B13-207A (the arrow denotes a light precipitation line); 7, 2B13-242C, a nonimmunoprecipitating, serum-neutralizing MAb control. In lanes 8 through 12, the antigen is [^{35}S]methionine-labeled BTV-10. Lanes: 8, BALB/c anti-BTV-13; 9, non-immune BALB/c serum; 10, 2B13-268D; 11, 4B13-207A; 12, 2B13-242C; 13, ^{14}C-labeled molecular weight standards.

no polyclonal antibody that has neutralizing activity to BTV-13 has detectable neutralizing activity to BTV-2 or immunoprecipitates its P2 polypeptide (Fig. 3). Obviously, if there were activity to both, BTV-2 and BTV-13 would not have been classified as separate serotypes.

With this study, we confirm the observation that P2 is the protein which induces the production of serum-neutralizing antibody to BTV (1, 6, 8, 9, 15) and show that a single MAb is able to neutralize both BTV-2 and BTV-13. Therefore, we deduce that BTV-2 and BTV-13 share a similar or identical epitope associated with serum neutralization and immunoprecipitation on P2. Whether this similarity rests in the primary amino acid sequence is not known and is unlikely in view of the RNA-RNA hybridization data presented by others. It is possible that the site in question is created by the tertiary structure of the protein which has resulted from other associated charges. We may be able to resolve this question shortly when we determine the sequence of the cDNA coding for P2 of BTV-2 and compare it with that of BTV-13. From this, one could also envision the development of a single vaccine for both serotypes, using either a synthetic peptide that is reactive to MAb 4B13-207A or its anti-idiotype.

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LITERATURE CITED

1. **Appleton, J., and G. J. Letchworth.** 1983. Monoclonal antibody analysis of serotype restricted and unrestricted bluetongue viral antigenic determinants. Virology **124**:286–299.
2. **Campbell, C. H., T. L. Barber, and M. M. Jochim.** 1978. Antigenic relationship of Ibaraki, bluetongue, and epizootic hemorrhagic disease viruses. Vet. Microbiol. **3**:15–22.
3. **Della-Porta, A. J., I. M. Parsonson, and D. A. McPhee.** 1985. Problems in interpretation of diagnostic tests due to cross reactions between orbiviruses and broad serological responses in animals, p. 445–453. *In* T. L. Barber and M. M. Jochim (ed.), Bluetongue and related orbiviruses. Alan R. Liss, Inc., New York.
4. **Fazekas de St. Groth, S., and D. Scheidegger.** 1980. Production of monoclonal antibodies: strategy and tactics. J. Immunol. Methods **35**:1–21.
5. **Huismans, H., and M. Cloete.** 1987. A comparison of different cloned bluetongue virus genome segments as probes for the detection of virus-specified RNA. Virology **158**:373–380.
6. **Huismans, H., and B. J. Erasmus.** 1981. Identification of the serotype specific antigens of bluetongue virus. Onderstepoort J. Vet. Res. **48**:51–58.
7. **Huismans, H., N. T. van der Walt, M. Cloete, and B. J. Erasmus.** 1983. The biochemical and immunological characterization of bluetongue virus outer capsid polypeptides, p. 165–172. *In* R. W. Compans and D. H. L. Bishop (ed.), Double stranded RNA viruses. Elsevier Biomedical Press, New York.
8. **Huismans, H., N. T. van der Walt, M. Cloete, and B. J. Erasmus.** 1987. Isolation of a capsid protein of bluetongue virus that induces a protective immune response in sheep. Virology **157**:172–179.
9. **Kahlon, J., K. Sugiyama, and P. Roy.** 1983. Molecular basis of bluetongue virus neutralization. J. Virol. **48**:627–632.
10. **Kearney, J. F., A. Radbruch, B. Llesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits construction of antibody secreting hybridoma cell lines. J. Immunol. **123**:1548–1550.
11. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
12. **Kessler, S. W.** 1976. Cell membrane antigen isolation with the staphylococcal protein A-antibody adsorbent. J. Immunol. **117**: 1482–1490.
13. **Kowalik, T. F., and K.-K. Li.** 1987. The genetic relatedness of United States prototype bluetongue viruses by RNA/RNA hybridization. Virology **158**:276–284.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Mecham, J. O., V. C. Dean, and M. M. Jochim.** 1986. Correlation of serotype specificity and protein structure of the five U.S. serotypes of bluetongue virus. J. Gen. Virol. **67**:2617–2624.
16. **Mulhern, F. J.** 1985. Economic impact of bluetongue and related orbiviruses in the western hemisphere, p. 445–453. *In* T. L. Barber and M. M. Jochim (ed.), Bluetongue and related orbiviruses. Alan R. Liss, Inc., New York.
17. **Roy, P., K. Sugiyama, C. D. Rao, J. Kusari, M. Purdy, and E. Collisson.** 1985. Molecular epidemiology of two U.S. orbiviruses: bluetongue virus and epizootic hemorrhagic disease virus, p. 589–595. *In* T. L. Barber and M. M. Jochim (ed.), Bluetongue and related orbiviruses. Alan R. Liss, Inc., New York.
18. **Samal, S. K., A. El-Hussein, F. R. Holbrook, B. J. Beatty, and R. F. Ramig.** 1987. Mixed infection of *Culicoides variipennis* with bluetongue virus serotypes 10 and 17: evidence for high frequency reassortment in the vector. J. Gen. Virol. **68**:2319–2329.
19. **Samal, S. K., C. W. Livingston, Jr., S. McConnell, and R. F. Ramig.** 1987. Analysis of mixed infection of sheep with bluetongue virus serotypes 10 and 17: evidence for genetic reassortment in the vertebrate host. J. Virol. **61**:1086–1091.
20. **Squire, K. R. E., R. Y. Chuang, L. F. Chuang, R. H. Doi, and B. I. Osburn.** 1986. Sequence relationships of United States prototype and wild-type bluetongue virus RNA genomes investigated by Northern blot hybridization analysis. Am. J. Vet. Res. **47**:53–60.
21. **Stott, J. L., R. D. Oberst, M. B. Channell, and B. I. Osburn.** 1987. Genome segment reassortment between two serotypes of bluetongue virus in a natural host. J. Virol. **61**:2670–2674.
22. **Taggart, R. T., and I. M. Samloff.** 1983. Stable antibody-producing murine hybridomas. Science **219**:1228–1230.
23. **Verwoerd, D. W., H. J. Els, E.-M. De Villiers, and H. Huismans.** 1972. Structure of the bluetongue virus capsid. J. Virol. **10**:783–794.