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Characterization of a Borna Disease Virus Glycoprotein, gp18

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Borna disease virus is a nonsegmented negative-strand RNA virus that causes neurologic disease in a wide variety of animal hosts. Here we describe identification and characterization of the first glycoprotein in this viral system. The 18-kDa glycoprotein, gp18, has been purified from infected rat brain. Isolation and microsequencing of this protein allowed identification of a 16.2-kDa open reading frame in the viral antigenome. Lectin binding and endoglycosidase sensitivity assays indicate that gp18 is an unusual N-linked glycoprotein.

Borna disease is an immune-mediated neurologic syndrome (31) caused by infection with a negative-strand RNA virus, Borna disease virus (BDV) (6, 8, 13, 14, 24). The natural host range of BDV includes birds, cats, sheep, and horses (25, 26, 27). Although the virus has not been recovered from human subjects, antibodies reactive with BDV proteins have been found in patients with bipolar depression, schizophrenia, or AIDS-related encephalopathy (4, 5, 35).

BDV has a unique tropism for specific brain regions. Viral nucleic acids and disease-specific proteins have been found in highest concentrations in the hippocampus and limbic circuits, prefrontal and cingulate cortices, and brainstem nuclei (10, 25, 40). Three BDV-specific proteins of 38/40, 24, and 14.5 kDa have been identified in infected cells and tissues (25, 44). cDNAs for the 38/40-kDa antigen (p40) (24, 30, 34) and the 24-kDa antigen (p23) (24, 45, 49) have been isolated, and complementary sequences to open reading frames (ORFs) for these proteins have been mapped to the 3′ end of the viral genome (8, 13). Using methods for isolation of the 14.5-kDa protein (36), we have purified a glycoprotein from BDV-infected rat brain that is encoded by a 429-nucleotide (nt) ORF located 3′ to ORF p23 on the viral antigenome. The protein is predicted to be 16.2 kDa; glycosylation results in a 1- to 2-kDa increase in molecular weight. This glycoprotein, gp18, is the first glycoprotein to be identified in the BDV system. Lectin binding and endoglycosidase sensitivity assays suggest that gp18 is an unusual N-linked glycoprotein.

MATERIALS AND METHODS

Infection of animals and cultured cells. Animals and cells were infected with BDV strain He/80 (20, 37). Newborn Lewis rats were infected by intracranial injection with 1.5×10^4 focus-forming units of BDV. Three weeks after infection, animals were sacrificed and brains were removed for isolation of BDV particles (9) or gp18. C6 cells and MDCK cells were persistently infected with BDV as described previously (11, 20). Monolayers of rabbit fetal glial cells were acutely infected by adding BDV at 1.0 focus-forming unit per cell to the culture medium (Dulbecco modified Eagle medium, 5% fetal calf serum; Gibco BRL).

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Protein purification and microsequencing. Protein was purified from infected cells and tissues by detergent-salt extraction by the method of Schädler et al. (36). For microsequencing, protein was cleaved with 10% cyanogen bromide in 75% formic acid (Sigma). Peptide fragments were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) on a Vydac C-18 column, using a trifluoroacetic acid-acetonitrile gradient. Sequence determinations were performed by automated Edman degradation on a Hewlett-Packard model G1000A protein sequencer.

Antibodies. Antibodies to purified gp18 were produced in 3-month-old BALB/c mice. Animals were injected subcutaneously with 5 μg of protein in Freund's complete adjuvant and boosted 3 weeks later with a subcutaneous injection of 3 μg of protein in Freund's incomplete adjuvant. For 6 weeks thereafter, at 2-week intervals, animals received intraperitoneal injections of 5 μg of protein in phosphate-buffered saline (PBS) with 5 μg of lipopolysaccharide (*Salmonella typhimurium*; Difco) (three injections). Blood was drawn every 2 weeks during weeks 7 through 28 for measurement of serum antibody titer to purified protein by Western blotting (immunoblotting). Antisera collected at week 28 were used for virus neutralization studies. Rabbit antisera to recombinant BDV p40 and p23 were used as controls (7).

Cloning and sequencing of cDNA encoding gp18. gp18-specific oligonucleotides were used to amplify full-length coding sequence for gp18 from two BDV-infected adult rat brain cDNA libraries (24, 30) as well as total cellular RNA (12) and poly(A)$^+$ RNA (2) extracted from infected rat brain. Reverse transcription (RT) was performed with an oligo(dT) primer and Superscript II (Gibco BRL, Life Technologies, Inc.). PCR was carried out with Ampli-Taq Stoffel fragment according to standard protocols (Perkin-Elmer) with the following primer pair: 5′-terminal *Xho*I-gp18 sense oligonucleotide (*Xho*I-gp18-S1), TCCTCGAGATGAATTCAAAACATTCCTATG (nt 1892 to 1914; *Xho*I restriction site indicated by underlining); and 3′-terminal gp18 antisense oligonucleotide (gp18-AS1), CTAAGGCCCTGAAGATCGAAT (nt 2301 to 2321). Products were purified by agarose gel electrophoresis using a USBioclean purification kit (U.S. Biochemical) and cloned into Bluescript SKII+ (Stratagene) prepared with 3′ T overhangs (28). A minimum of three independent clones from each template source was sequenced on both strands by the dideoxynucleotide chain termination method using bacteriophage T7 DNA polymerase (Sequenase; U.S. Biochemical).

The plasmid resulting from amplification of neonatally infected rat brain RNA was named pBDV-gp18.

In vitro transcription, translation, and cotranslational processing. Plasmid clones pBDV-gp18 and pBDV-23 (43) linearized with *Eco*RI were used as templates for in vitro synthesis of capped RNA transcripts. Transcription products or *Saccharomyces cerevisiae* α-factor mRNA (control for glycosylation) were translated in vitro by using nuclease-treated rabbit reticulocyte lysates (Promega Corp.) in the presence of [^{35}S]methionine (Amersham). Cotranslational processing was assessed by in vitro translation using reticulocyte lysates supplemented with canine microsomal membranes (Promega). Transcription, translation, and cotranslational processing studies were performed according to the manufacturer's protocols. Translation products were immunoprecipitated with mouse anti-gp18 serum and then size fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (13% gel) (21) for autoradiographic analysis. Methods for immunoprecipitation and autoradiography have been described elsewhere (24).

Protein gel electrophoresis and immunoblotting. Proteins were size fractionated by SDS-PAGE (12% gel) and then transferred to Immobilon-N membranes (Millipore Corp.) (46). Primary antisera for immunoblotting were from rats chronically infected with BDV (day 100 after intracranial infection) or mice immunized with purified gp18. The secondary antibody was alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (Sigma); the substrate was Western Blue (Promega Corp.).

Carbohydrate analysis. Purified protein was size fractionated by SDS-PAGE (13% gel) and then either silver stained for detection of protein (18) or carbohydrate (48) or transferred to Immobilon-N membranes (Millipore) for lectin staining. The carbohydrate composition of immobilized protein was determined by using a DIG Glycan Differentiation Kit (Boehringer Mannheim) and peroxidase-labeled *Bandeiraea simplicifolia* agglutinins I and II (BS-I and BS-II; Sigma). The substrate for peroxidase was 4-chloro-1-naphthol (Pierce). Glycosidase digests of native and denatured protein (incubated for 5 min at 100°C in 0.01% SDS) were performed according to the manufacturer's protocols, using the following endoglycosidases: endoglycosidase F and *N*-glycosidase F; *O*-glycosidase; *N*-glycosidase F; endoglycosidase F, *N*-glycosidase free; endoglycosidase H; and endo-β-galactosidase (Boehringer Mannheim).

RESULTS

Isolation of gp18. Protein was isolated from neonatally infected rat brain, acutely infected rabbit fetal glial cells (two passages), persistently infected C6 cells, and persistently infected MDCK cells, using the method of Schädler et al. (36). The purity of the protein was confirmed by silver staining of the protein after SDS-PAGE (data not shown). The quantity of protein was estimated in silver-stained gels by using lysozyme standards. Typical yields were 5 μg of protein from one neonatally infected rat brain and 2 μg of protein from 10^8 infected cultured cells. Protein from neonatally infected rat brain was used for microsequencing, carbohydrate analysis, and immunization of mice.

Protein and nucleic acid sequence analysis. Direct microsequencing of gp18 was not possible because of a blocked amino terminus; thus, to allow analysis, the protein was cleaved with cyanogen bromide. Sequencing of the cleavage mixture indicated the presence of three N termini. From the mixture, two peptides (peptides 1 and 3; Fig. 1) were isolated by RP-HPLC and sequenced individually, allowing inference of a third sequence (peptide 2; Fig. 1) by subtraction. Peptide sequences were used as probes to search ORFs located on the BDV antigenome. The peptide sequences obtained from the purified gp18 mapped to a 429-nt ORF (ORF gp18) on the viral antigenome that predicts a 142-amino-acid protein with a molecular weight of 16,244 (Fig. 1).

Genomic sequence corresponding to the gp18 ORF was used to design probes and primers for identifying mRNA encoding gp18. In each of two cDNA libraries prepared from BDV-infected adult rat brain poly(A)$^+$ RNA (24, 30), 100,000 recombinants were screened by hybridization with a 271-bp *Hinc*II-*Hin*fI restriction fragment from pTB-BDV 5.82 (nt 2062 to 2333 in the viral genome) (8). These libraries were also screened by PCR using the 5′-terminal *Xho*I-gp18 sense primer (nt 1892 to 1914) and oligo(dT). Total cellular and poly(A)$^+$ RNAs extracted from persistently infected C6 cells, BDV-infected adult rat brain, or 3-week-old neonatally infected rat brain (the peak time point for in vivo expression of gp18) were subjected to RT-PCR using oligo(dT) in combination with the 5′-terminal *Xho*I-gp18 sense primer. No gp18-specific transcript corresponding to the size of ORF gp18 was obtained in these experiments. In contrast, use of the 5′-terminal *Xho*I-gp18 sense primer in combination with a 3′-terminal gp18 antisense primer (nt 2301 to 2321) allowed amplification of gp18 sequences from any of these sources by RT-PCR. In spite of variability at the nucleic acid level, the predicted amino acid sequence obtained from the different sources was the same as for strain V genomic sequence, with the exception of a single exchange in position 108 (E→D) (Fig. 1).

Characterization of gp18 as a glycoprotein. Purified gp18 was size fractionated by SDS-PAGE. Modified silver staining revealed the presence of carbohydrate; thus, fractionated protein was blotted onto Immobilon-N membranes to determine the presence of individual saccharides through lectin binding studies. Binding was observed with *Cancanavalia ensiformis* agglutinin (ConA), wheat germ agglutinin, *Datura stramonium* agglutinin, BS-I, and BS-II but not with *Galanthus nivalis* agglutinin, *Sambucus nigra* agglutinin, *Maackia amurensis* agglutinin, and peanut agglutinin (Fig. 2). This staining pattern was consistent with the presence of *N*-acetylglucosamine, *N*-acetylgalactosamine, mannose, and galactose. In addition, native and denatured proteins were digested with specific endoglycosidases, size fractionated by SDS-PAGE, and then stained to assess molecular weight shift and presence or absence of carbohydrate. Treatment with *O*-glycosidase or endoglycosidase H had no effect (data not shown). In contrast, treatment with endoglycosidase F and *N*-glycosidase F resulted in a loss of 1 to 2 kDa (Fig. 3) and abrogation of lectin staining with ConA (data not shown). Treatment with endoglycosidase F (*N*-glycosidase free) or endo-β-galactosidase also resulted in a loss of 1 to 2 kDa (Fig. 3).

In vitro transcription, translation, and processing of gp18. With linearized pBDV-gp18 used as a template, gp18 RNA was transcribed and translated in vitro in either the presence or absence of canine microsomal membranes. The gp18 RNA directed translation of two proteins of 16 and 18 kDa that were recognized by monospecific murine antiserum to purified gp18. Translation in the presence of microsomal membranes led to an increase in the relative proportion of the 18-kDa protein. Treatment with endoglycosidase F resulted in loss of the 18-kDa protein species (Fig. 4A). Glycosylation of the 18-kDa species was also shown by lectin binding studies performed after translation products were size fractionated by SDS-PAGE and transferred to membranes. The 18-kDa protein was recognized by ConA, whereas the 16-kDa protein did not bind

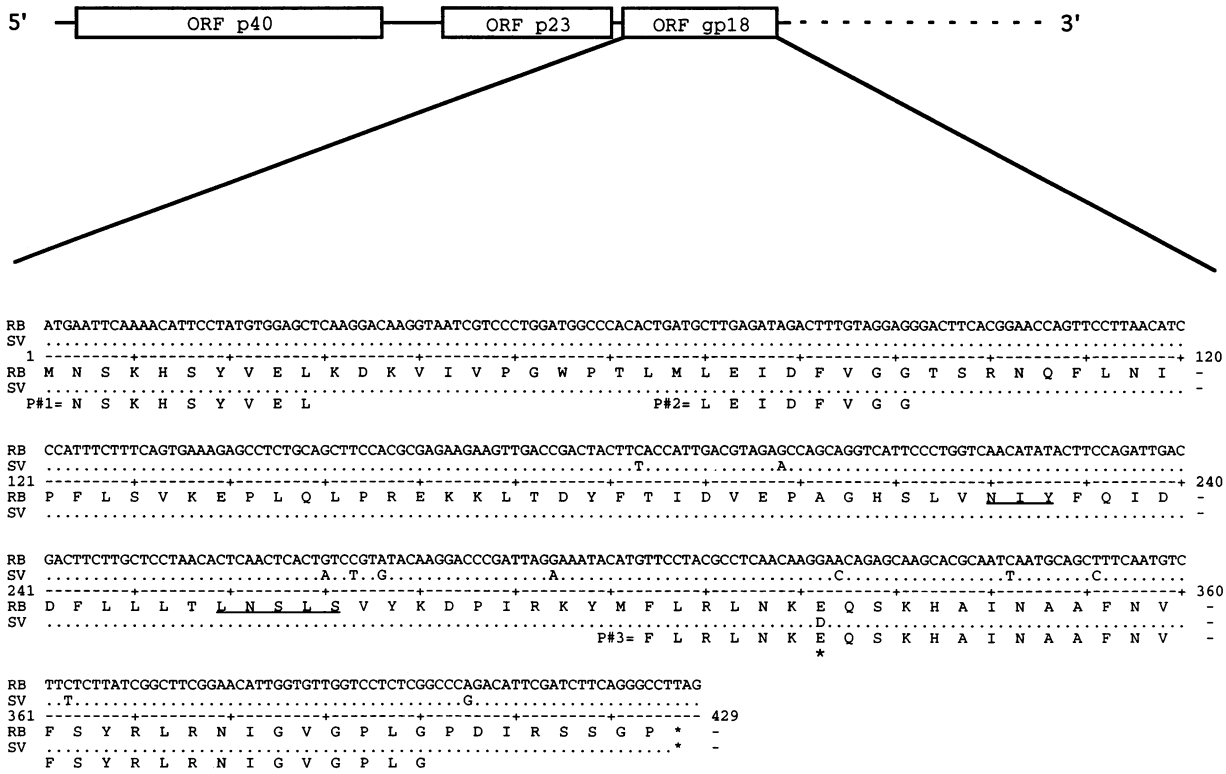

FIG. 1. Sequence of ORF gp18. The diagram shows the location of ORF gp18 on the viral antigenome (5′-3′) relative to ORFs p40 and p23 (boxes). ORF gp18 sequences were from Oligo/TL cells infected with BDV strain V (SV) and rat brain infected with BDV He/80 (RB). Peptide sequences (P#1, P#2, and P#3) were obtained by microsequencing of purified protein from He/80-infected rat brain. Periods indicate identical nucleotide or amino acid sequences. Variable amino acid residues (large asterisk) and stop codons (small asterisks) are indicated. Underlining indicates potential glycosylation sites.

ConA (Fig. 4B). Modification of translated protein by the microsomal membranes was specific for gp18. Translation of RNA encoding BDV p23, which encodes a potential N-glycosylation site (amino acids 53 to 55), included as a negative control for in vitro glycosylation, was not influenced by the presence of microsomal membranes (Fig. 4A).

DISCUSSION

We have isolated and partially characterized a BDV glycoprotein with unusual properties. This protein, previously reported as 14.5 kDa (36), is 16.2 kDa prior to carbohydrate modification and ≈18 kDa after glycosylation. Though no

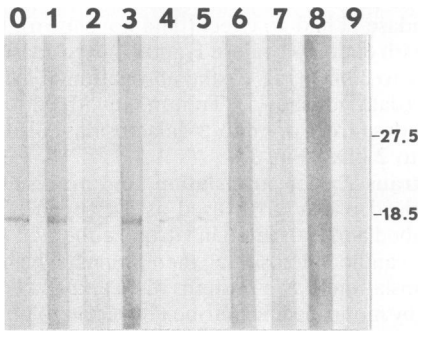

FIG. 2. Glycan determination of gp18. gp18 isolated from infected rat brain was size fractionated by SDS-PAGE (12% gel) then transferred to an Immobilon-N membrane for lectin staining (see Materials and Methods). Lanes: 0, protein detection by mouse anti-gp18 serum; 1, ConA; 2, wheat germ agglutinin; 3, *D. stramonium* agglutinin; 4, BS-I; 5, BS-II; 6, G. *nivalis* agglutinin; 7, *S. nigra* agglutinin; 8, *M. amurensis* agglutinin; 9, peanut agglutinin. Positions of molecular weight markers are shown in kilodaltons at the right.

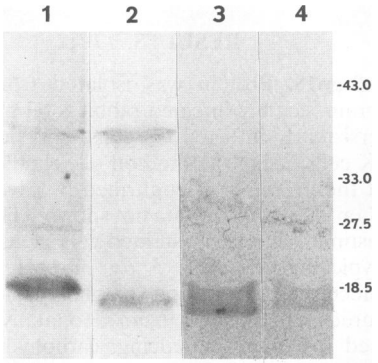

FIG. 3. gp18 is sensitive to endoglycosidases. gp18 isolated from infected rat brain was treated with either buffer alone or endoglycosidase. Protein was size fractionated by SDS-PAGE (13% gel) and detected by silver staining. Lanes: 1, buffer; 2, endoglycosidase F plus *N*-glycosidase F; 3, endoglycosidase F (*N*-glycosidase free); 4, endo-β-galactosidase. Positions of molecular weight markers are shown in kilodaltons at the right.

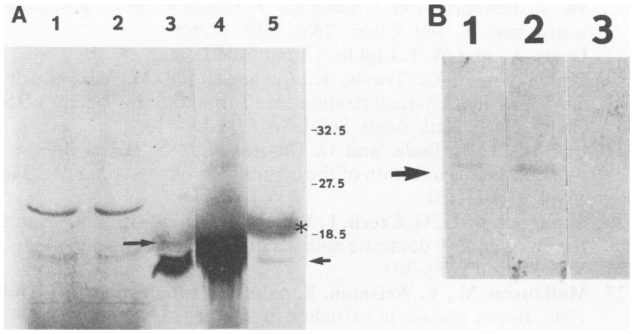

FIG. 4. In vitro transcription, translation, and cotranslational processing of gp18. RNA transcripts were synthesized from pBDV-23 (a nonglycosylated BDV protein control) or pBDV-gp18 and translated in vitro by using rabbit reticulocyte lysates in either the absence or presence of canine microsomal membranes. [^{35}S]methionine-labeled translation products were immunoprecipitated with antisera to p23 or gp18 and protein A-Sepharose and then size fractionated by SDS-PAGE (13% gel) for autoradiography (A) or transferred to Immobilon-N membranes for ConA lectin staining (B). Translated gp18 in lane 5 of panel A and lane 3 of panel B was incubated with endoglycosidase F plus *N*-glycosidase F prior to SDS-PAGE. (A) Lanes: 1, pBDV-23 RNA; 2, pBDV-23 RNA plus microsomal membranes; 3, pBDV-gp18 RNA; 4, pBDV-gp18 RNA plus microsomal membranes; 5, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases. The long arrow indicates the position of glycosylated protein (lanes 3 and 4); the short arrow indicates the position of protein after treatment with endoglycosidase F plus *N*-glycosidase F (lane 5). The asterisk indicates nonspecific background signal (lane 5). Positions of molecular weight markers are shown in kilodaltons at the right. (B) Lanes: 1, pBDV-gp18 RNA; 2, pBDV-gp18 RNA plus microsomal membranes; 3, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases.

classical sites for N linkage (N-x-S/T [29]) are found in the gp18 sequence, the protein is readily modified in vitro in the presence of a microsomal membrane system capable of N glycosylation (16, 22). In addition, gp18 is sensitive to *N*-glycosidase F, an enzyme which cleaves between asparagine and *N*-acetylglucosamine (33, 41). These findings indicate that gp18 is N glycosylated at a nonclassical site. One potential site is N-I-Y (amino acids 74 to 76). The presence of a hydroxyl amino acid (T or S) or cysteine in position +2 (N-x-T/S or C) has been proposed as essential for hydrogen bond donor function in N glycosylation (3). It is possible that tyrosine (Y), another hydroxyl amino acid in position +2, could serve as a hydrogen bond donor in gp18. A second potential site for N glycosylation is L-N-S-L-S (amino acids 87 to 91), which is similar to S-N-S-G-phosphorylated S, the site for N glycosylation in a glycopeptide from hen yolk phosvitin (39).

gp18 is sensitive to endoglycosidase F, an enzyme that cleaves after the N-linked *N*-acetylglucosamine in high mannose-, biantennary hybrid-, and biantennary complex-type oligosaccharides (41, 42). The protein is not sensitive to endoglycosidase H, an enzyme which cleaves after the N-linked *N*-acetylglucosamine in high-mannose- and most hybrid-type oligosaccharides but does not cleave complex-type oligosaccharides (47). Lectin staining using *G. nivalis* agglutinin shows no evidence of terminal mannose characteristic for hybrid- and high-mannose-type glycosylation. In contrast, staining with ConA (mannose, *N*-acetylglucosamine, branched trimannosyl core) (32), wheat germ agglutinin (*N*-acetylglucosamine), and BS-II (terminal *N*-acetylglucosamine) (15) indicates the presence of terminal *N*-acetylglucosamine and internal mannose. Thus, there is evidence from the pattern of endoglycosidase sensitivity and lectin staining that gp18 is likely to be a biantennary complex-type glycoprotein.

gp18 is sensitive to endo-β-galactosidase. This enzyme cleaves between galactose and either *N*-acetylglucosamine or galactose when these saccharides occur in unbranched sequence (38). The presence of galactose was confirmed by BS-I lectin binding (Fig. 2). The presence of both *N*-acetylglucosamine and galactose was confirmed by high-performance anion-exchange chromatography with pulsed amperometric detection (data not shown). The combination of *N*-acetylgalactosamine and galactose is usually found in O-linked carbohydrates (19). Though it is possible that gp18 is both N and O glycosylated, *N*-acetylgalactosamine has also been reported to occur in complex-type N-linked glycosylation (19).

We did not detect a monocistronic ≈429-nt mRNA for gp18 by PCR using oligo(dT), a 5′ sense primer, and template from a variety of sources, including infected cell lines and rat brain. In contrast, a 429-nt gp18 cDNA was readily amplified by using gene-specific primers and total RNA or poly(A)$^+$ RNA as a template. Northern (RNA) hybridization experiments with gp18-specific probes using total RNA or poly(A)$^+$ RNA from infected cells or rat brain detected only 1.5-, 2.8-, 3.5-, 6.1-, and 7.1-kb transcripts (23). Recent experiments confirmed that the 1.5- and 2.8-kb RNAs can serve as templates for in vitro translation of the gp18 (data not shown). These data suggest that gp18 is likely to be translated from one or more of the larger RNA transcripts.

The role of gp18 in the BDV life cycle remains to be determined. Though the virus has not been characterized morphologically, genetic analysis has characterized BDV as a member of the order *Mononegavirales* (8, 13). In nonsegmented, negative-strand RNA viruses, the third gene usually directs expression of a matrix protein. Matrix proteins in members of the order *Mononegavirales* are not known to be glycosylated; however, glycosylated matrix proteins that resemble gp18 in size and pI (≈10) have been found in other viral systems (e.g., E1 in coronaviruses [1]). Preliminary observations suggest that gp18 is present on the surface of the viral envelope. Monospecific antisera and monoclonal antibodies to gp18 precipitated viral particles and had neutralizing activity. In contrast, antibodies to p40 and p23 did not precipitate viral particles or neutralize infectivity (18). Preincubation of primary rabbit fetal glia (cells highly susceptible to BDV) with gp18 prevented infection. No such effect was observed with either p40 or p23. Last, both gp18 and BDV particles bound to a ≈100-kDa membrane protein present in cells susceptible to infection. Future efforts will be directed toward determining the nature and significance of interactions between gp18 and host cell proteins.

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REFERENCES

1. **Armstrong, J., H. Niemann, S. Smeekens, P. Rottier, and G. Warren.** 1984. Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. Nature (London) **308:**751–752.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69:**1408–1412.
3. **Bause, E., and G. Legler.** 1981. The role of the hydroxy amino acid in the triplet sequence Asn-Xaa-Thr(Ser) for the N-glycosylation step during glycoprotein biosynthesis. Biochem. J. **195:**639–644.
4. **Bode, L., R. Ferszt, and G. Czech.** 1993. Borna disease virus infection and affective disorders in man. Arch. Virol. Suppl. **7:** 159–167.
5. **Bode, L., S. Riegel, H. Ludwig, J. Amsterdam, W. Lange, and H. Koprowski.** 1988. Borna disease virus-specific antibodies in patients with HIV infection and with mental disorders. Lancet **ii:**689.
6. **Briese, T., J. C. de la Torre, A. Lewis, H. Ludwig, and W. I. Lipkin.** 1992. Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. Proc. Natl. Acad. Sci. USA **89:**11486–11489.
7. **Briese, T., and W. Lipkin.** Unpublished data.
8. **Briese, T., A. Schneemann, A. J. Lewis, Y.-S. Park, S. Kim, and W. I. Lipkin.** 1994. Genomic organization of Borna disease virus. Proc. Natl. Acad. Sci. USA **91:**4362–4366.
9. **Carbone, K., C. Duchala, J. Griffin, A. Kincaid, and O. Narayan.** 1987. Pathogenesis of Borna disease in rats: evidence that intraaxonal spread is the major route for virus dissemination and the determination for disease incubation. J. Virol. **61:**3431–3440.
10. **Carbone, K., T. Moench, and W. I. Lipkin.** 1991. Borna disease virus replicates in astrocytes, Schwann cells and ependymal cells in persistently infected rats: location of viral genomic and messenger RNAs by in situ hybridization. J. Neuropathol. Exp. Neurol. **50:** 205–214.
11. **Carbone, K. M., S. A. Rubin, A. M. Sierra-Honigman, and H. M. Lederman.** 1993. Characterization of a glial cell line persistently infected with Borna disease virus (BDV): influence of neurotrophic factors on BDV protein and RNA expression. J. Virol. **67:** 1453–1460.
12. **Chirgwin, J. J., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5299.
13. **Cubitt, B., C. Oldstone, and J. C. de la Torre.** 1994. Sequence and genome organization of Borna disease virus. J. Virol. **68:**1382–1996.
14. **de la Torre, J., K. Carbone, and W. I. Lipkin.** 1990. Molecular characterization of the Borna disease agent. Virology **179:**853–856.
15. **Ebisu, S., and I. J. Goldstein.** 1978. *Bandeira simplicifolia* lectin II. Methods Enzymol. **50:**350–354.
16. **Gahmberg, C. G., M. Jokinen, K. K. Karhi, O. Kämpe, P. A. Peterson, and L. C. Andersson.** 1983. Glycophorin A: in vitro biogenesis and processing, p. 281–297. *In* S. Fleischer and B. Fleischer (ed.), Biomembranes. Academic Press, New York.
17. **Hames, D.** 1990. One-dimensional polyacrylamide gel electrophoresis, p. 1–139. *In* B. D. Hames and D. Rickwood (ed.), Gel electrophoresis of proteins: a practical approach. IRL Press, Oxford.
18. **Hatalski, C. G., S. Kliche, T. Briese, L. Stitz, and W. I. Lipkin.** Neutralizing antibodies in Borna disease virus infected rats. Submitted for publication.
19. **Hayes, B. K., and A. Varki.** 1993. The biosynthesis of oligosaccharides in intact golgi preparations from rat liver. J. Biol. Chem. **268:**16170–16178.
20. **Herzog, S., and R. Rott.** 1980. Replication of Borna disease virus in cell cultures. Med. Microbiol. Immunol. **168:**153–158.
21. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. J. Mol. Biol. **80:**575–581.
22. **Lau, J. T. Y., J. K. Welply, P. Shenbagamurthi, F. Naider, and W. J. Lennarz.** 1983. Substrate recognition by oligosaccharyl transferase. J. Biol. Chem. **258:**15255–15260.
23. **Lewis, A., and W. I. Lipkin.** Unpublished data.
24. **Lipkin, W. I., G. Travis, K. Carbone, and M. Wilson.** 1990. Isolation and characterization of Borna disease agent cDNA clones. Proc. Natl. Acad. Sci. USA **87:**4184–4188.
25. **Ludwig, H., L. Bode, and G. Gosztonyi.** 1988. Borna disease: a persistent virus infection of the central nervous system. Prog. Med. Virol. **35:**107–151.
26. **Lundgren, A.-L., G. Czech, L. Bode, and H. Ludwig.** 1993. Natural Borna disease in domestic animals other than horses and sheep. J. Vet. Med. **40:**298–303.
27. **Malkinson, M., Y. Weisman, E. Ashash, L. Bode, and H. Ludwig.** 1993. Borna disease in ostriches. Vet. Rec. **133:**304.
28. **Marchuk, D., M. Drumm, A. Saulino, and F. S. Collins.** 1990. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acid Res. **19:**1154.
29. **Marshall, R. D.** 1972. Glycoproteins. Annu. Rev. Biochem. **41:** 673–702.
30. **McClure, M. A., K. J. Thibault, C. G. Hatalski, and W. I. Lipkin.** 1992. Sequence similarity between Borna disease virus p40 and a duplicated domain within the paramyxo- and rhabdovirus polymerase proteins. J. Virol. **66:**6572–6577.
31. **Narayan, O., S. Herzog, K. Frese, H. Scheefers, and R. Rott.** 1983. Behavorial disease in rats caused by immunopathological responses to persistent Borna virus in the brain. Science **220:**1401–1403.
32. **Ogata, S., T. Muramatsu, and A. Kobata.** 1975. Fractionation of glycopeptides by affinity column chromatography on concanavalin A-sepharose. J. Biochem. **78:**687–696.
33. **Plummer, T. H., H. J. Elder, S. Alexander, A. W. Phelan, and A. L. Tarentino.** 1984. Demonstration of peptide: N-glycosidase F activity in end-β-N-acetylglucosaminidase F preparations. J. Biol. Chem. **259:**10700–10704.
34. **Pyper, J. M., J. A. Richt, L. Brown, R. Rorr, O. Narayan, and J. E. Clements.** 1993. Genomic organization of the structural proteins of Borna disease virus revealed by a cDNA clone encoding the 38-kDa protein. Virology **195:**229–238.
35. **Rott, R., S. Herzog, B. Fleischer, A. Winokur, J. Amsterdam, W. Dyson, and H. Koprowski.** 1985. Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. Science **228:**755–756.
36. **Schädler, R., H. Diringer, and H. Ludwig.** 1985. Isolation and characterization of a 14500 molecular weight protein from brains and tissue cultures persistently infected with Borna disease virus. J. Gen. Virol. **66:**2479–2484.
37. **Schneider, P. A., T. Briese, W. Zimmermann, H. Ludwig, and W. I. Lipkin.** 1994. Sequence conservation in field and experimental isolates of Borna disease virus. J. Virol. **68:**63–68.
38. **Scudder, P., P. Hanfand, K. Uemura, and F. T.** 1984. Endo-β-D-galactosidase of Bacteroides fragilis and Escherichia freundii hydrolyze but not branched oligosaccharides domaims of glycolipids of the neolacto series. J. Biol. Chem. **259:**6586–6592.
39. **Shainkin, R., and G. Perlmann.** 1971. Phosvitin, a phospoglycoprotein. J. Biol. Chem. **246:**2278–2284.
40. **Solbrig, M. V., G. F. Koob, and W. I. Lipkin.** 1992. Borna disease in rats: a new model for virus-induced movement disorders, abstr. 10. Abstr. 1992 Am. Acad. Neurol. Annu. Meet.
41. **Tarentino, A. L., C. M. Gomez, and T. H. Plummer.** 1985. Deglycosylation of asparagine-linked glycans by peptide: N-glycosidase F. Biochem. **24:**4665–4671.
42. **Tarentino, A. L., and T. H. Plummer.** 1994. Enzymatic deglycosylation of asparagine-linked glycans: purification, properties, and specificity of oligosaccharide-cleaving enzymes from Flavobacterium meningosepticum. Methods Enzymol. **230:**44–57.
43. **Thibault, K. J.** 1992. M.S. thesis. University of California, Irvine.
44. **Thiedemann, N., P. Presek, R. Rott, and L. Stitz.** 1992. Antigenic relationship and further characterization of two major Borna disease virus-specific proteins. J. Gen. Virol. **73:**1057–1064.
45. **Thierer, J., H. Riehle, O. Grebenstein, T. Binz, S. Herzog, N. Thiedemann, L. Stitz, R. Rott, F. Lottspeich, and H. Niemann.** 1992. The 24K protein of Borna disease virus. J. Gen. Virol. **73:** 413–416.

46. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
47. **Trimbel, R. B., and F. Maley.** 1984. Optimizing hydrolysis of N-linked high-mannose oligosaccharides by endo-β-N-acetylglucosaminidase. Anal. Biochem. **141:**515–522.
48. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119:**115–119.
49. **VandeWoude, S., J. Richt, M. Zink, R. Rott, O. Narayan, and J. Clements.** 1990. A Borna virus cDNA encoding a protein recognized by antibodies in humans with behavioral diseases. Science **250:**1276–1281.