Molecular Biology of Borna Disease Virus

T. BRIESE[1,2], W.I. LIPKIN[1,3], and J.C. DE LA TORRE[4]

1 Introduction 1
2 Genetics 2
2.1 Characterization of BDV Nucleic Acids 2
2.2 BDV Contains Genomic RNA of Negative Polarity 5
3 Transcription 6
3.1 Transcription of BDV Occurs in the Nucleus of the Infected Cell 6
4 Proteins 8
4.1 Borna Disease-Specific Proteins 8
4.2 Analysis of Cloned Viral Proteins 9
5 Concluding Remarks 11
References 13

1 Introduction

Originally described in the early nineteenth century as a fatal encephalitis in horses, Borna disease (BD) has become an extraordinarily valuable model for the study of both molecular mechanisms and biological consequences of persistent virus infection in the CNS (NICOLAU and GALLOWAY 1928; ZWICK 1939, this volume). BD is an immune-mediated neurologic syndrome characterized by behavioral abnormalities, meningeal and parenchymal inflammatory cell infiltrates in the brain, and the accumulation of disease-specific antigen in limbic system neurons (JOEST and DEGEN 1911; SEIFRIED and SPATZ 1930; LUDWIG et al. 1988; RICHT et al. 1992). As a natural infection, BD has only been confirmed to occur in horses and sheep, but experimentally it can be transmitted to an extraordinary wide range of host species, including birds, rodents and nonhuman primates (ZWICK et al. 1926; NICOLAU and GALLOWAY 1928; ZWICK 1939; MATTHIAS 1954; NITZSCHKE 1963; HEINIG 1969; ANZIL et al. 1973; LUDWIG et al. 1973; METZLER et al. 1976; LUDWIG and THEIN

[1] Department of Neurology, University of California, Irvine, CA 92717, USA
[2] Institute of Virology, Freie Universität Berlin, Nordufer 20, 13353 Berlin, Germany
[3] Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717, USA
[4] Department of Neuropharmacology, Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

H. Koprowski et al. (eds.), *Borna Disease*
© Springer-Verlag Berlin Heidelberg 1995

1977; SPRANKEL et al. 1978; STITZ et al. 1980; HIRANO et al. 1983, NARAYAN et al. 1983; GOSZTONYI and LUDWIG 1984; KAO et al. 1984; LUDWIG et al. 1985; WAELCHLI et al. 1985; RICHT et al. 1992). Serological data indicate that the host range may even extend to humans, although no infectious material has been isolated from human subjects (AMSTERDAM et al. 1985; ROTT et al. 1985, 1991; BODE et al. 1988, 1992).

Despite remarkable progress achieved during the past decade in understanding the pathogenesis of BD, until quite recently little was known about the etiologic agent for this disease (LUDWIG et al. 1988; DUCHALA et al. 1989). Biochemical studies indicated that BD is induced by an agent which is sensitive to detergents, organic solvents and UV light (NICOLAU and GALLOWAY 1928; DANNER and MAYR 1979; DUCHALA et al. 1989). In addition, a size of 80–125 nm for the agent was estimated, based on filtration experiments (ELFORD and GALLOWAY 1933; DANNER and MAYR 1979). These results indicated that BD is likely to be caused by a conventional, enveloped virus, the Borna disease virus (BDV). However, such features as noncytopathic replication, low titers of infectious material and tight cell association hampered isolation and further characterization of this agent (LUDWIG et al. 1988). The detection of BDV relied primarily upon indirect, immunologic methods (WAGNER et al. 1968; GOSZTONYI and LUDWIG 1984; PAULI et al. 1984). BD induces the production of specific proteins, the "s-antigen," which elicit a strong immune response (VON SPROCKHOFF 1956; LUDWIG and BECHT 1977). Polyclonal serum antibodies and oligoclonal antibodies in the CNS directed against s-antigen components of 38/40 kDa and 24 kDa have been characterized (LUDWIG et al. 1977, 1988). Immunohistochemical analysis indicated that the agent is highly neurotropic and spreads intra-axonally (KREY et al. 1979; CARBONE et al. 1987; MORALES et al. 1988). Definitive proof of the viral nature of the agent came only after application of molecular genetic approaches, which led to the isolation of BDV-specific cDNAs and a partial characterization of this virus (LIPKIN et al. 1990; VANDEWOUDE et al. 1990).

In this chapter, we summarize the present knowledge of the molecular biology of Borna disease virus, the causative agent of Borna disease.

2 Genetics

2.1 Characterization of BDV Nucleic Acids

The isolation of BDV-specific cDNAs by using subtractive cloning procedures has provided both direct evidence for an infectious basis for BD and a battery of tools for molecular characterization of BDV. Two classes of cDNAs corresponding to each of the two major antigens known to be specific for BD were isolated from BDV-infected rat brain: one class corresponding to a 40 kDa protein, open reading frame (ORF) p40, the other to a 24 kDa protein, ORF p24 (LIPKIN et al. 1990). cDNAs

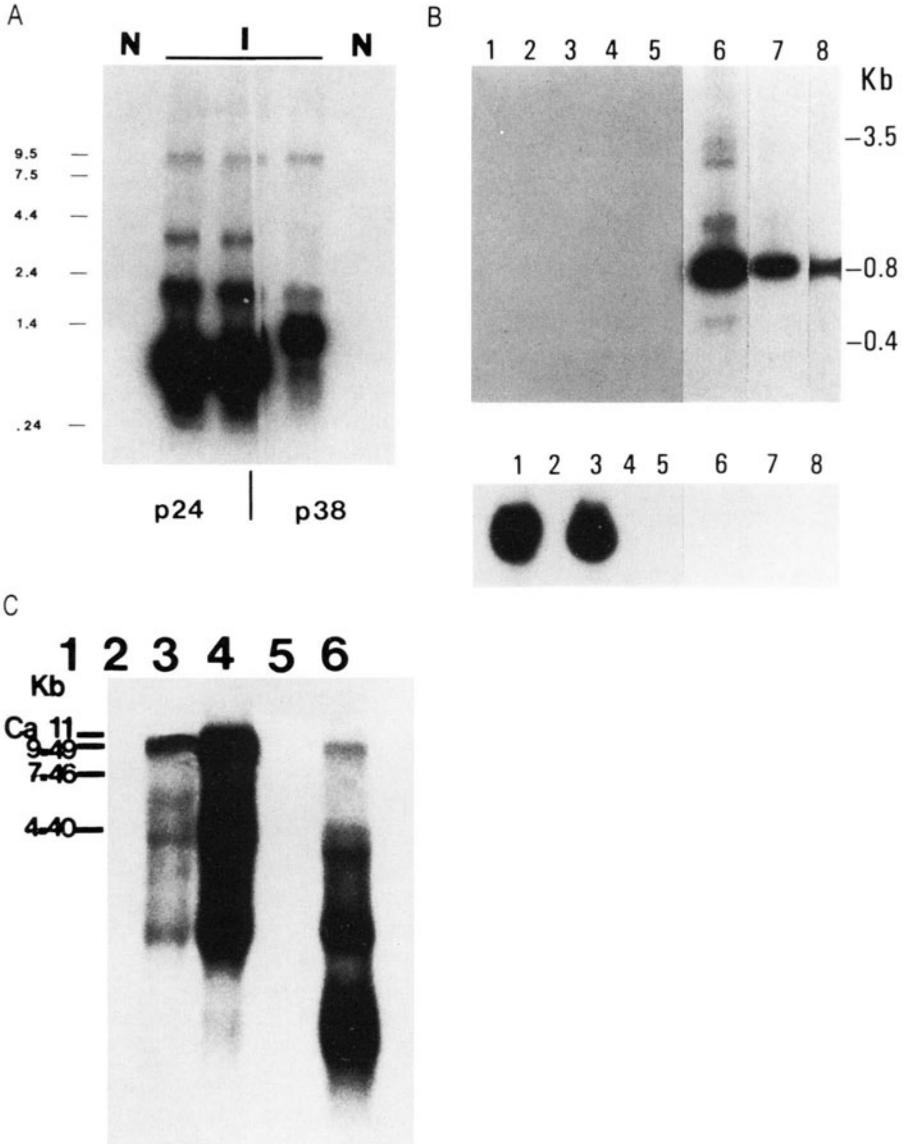
of the latter class were also isolated from BD-infected tissue culture cells through subtractive cloning or through oligonucleotide screening using sequences deduced from direct amino acid sequencing of purified BD antigen (VANDE WOUDE et al. 1990; THIERER et al. 1992).

Northern blot experiments using these cDNA probes revealed RNAs of 0.8–1.2 kb, 2.1 kb, 3.5 kb and approximately 8.5 kb, exclusively present in extracts obtained from BDV-infected material (Fig. 1) (LIPKIN et al. 1990; DE LA TORRE et al. 1990; VANDEWOUDE et al. 1990). In Southern hybridization studies, the cDNA probes did not hybridize to genomic DNA, ruling out the possibility that the cloned sequences were of cellular origin (LIPKIN et al. 1990; VANDEWOUDE et al. 1990). Accordingly, the pattern of BDV nucleic acid signal found in in situ hybridization studies of BD rat brain was not evenly distributed over all cells, but was instead consistent with the well characterized distribution of BD antigen in infected rat brain (see also Gosztonyi and Ludwig, this volume). Hybridization signal was found in layer 4 and 5 cortical neurons and in brainstem, with higher density over limbic structures including thalamus and sectors CA3, CA4 of hippocampus (LIPKIN et al. 1990; CARBONE et al. 1991; RICHT et al. 1991). Because of this hetero-geneous distribution it was necessary to address the question whether BDV is a DNA or RNA virus, or a retrovirus in homogeneously infected cell populations by using persistently BDV-infected cell lines. Immunofluorescence as well as in situ hybridization analysis showed that in the MBV line, a persistently BDV-infected cell line derived from MDCK cells, all the cells were infected (DE LA TORRE et al. 1990). BDV sequences were not detected in genomic or episomal DNA obtained from these cells, using conditions that allowed the detection of 0.5 gene copies per cell genome equivalent (Fig. 1) (DE LA TORRE et al. 1990). These results demonstrated that BDV is neither a DNA virus nor a retrovirus (DE LA TORRE et al. 1990; VANDE WOUDE et al. 1990). The sensitivity of the BDV-specific nucleic acids to pancreatic RNase digestion led to the hypothesis that BDV is a single-stranded RNA virus (DE LA TORRE et al. 1990).

Hybrid arrest experiments suggested that the two classes of cDNA direct the synthesis of two proteins specific for BD, the 40 kDa and the 24 kDa antigens (LIPKIN et al. 1990). Consequently, individual transcripts can be detected in northern hybridization experiments depending on the cDNA probe used: the ORF p40 probe reveals a 1.2 kb signal, while the ORF p24 probe reveals a 0.8 kb signal (Fig. 1). The largest RNA, of approximately 8.5 kb, and the 2.1 kb RNA cross-hybridize with the both probes, whereas the 3.5 kb RNA hybridizes only with the ORF p24 probe. The use of strand-specific RNA and oligonucleotide probes demonstrated a positive polarity for the RNAs of 0.8, 1.2, 2.1 and 3.5 kb (LIPKIN et al. 1990; VANDEWOUDE et al. 1990; Lewis and Lipkin, unpublished). The 0.8, 1.2, 2.1 and 3.5 kb RNAs are polyadenylated (DE LA TORRE et al. 1990; VANDEWOUDE et al. 1990; MCCLURE et al. 1992; THIERER et al. 1992). Whereas an mRNA function for the 0.8 and 1.2 kb RNAs has been established, the role of the 2.1 and 3.5 kb RNAs is not yet clear.

The findings regarding the largest identified RNA species, the putative genomic RNA of BDV, are a subject of controversy. First, VANDEWOUDE et al. (1990)

reported different sizes of 10.5 kb and 8.7 kb for the largest BDV RNA species when derived from BD rat brain or infected MDCK cells, respectively, suggesting that this difference may reflect the presence of a defective genome in the persistently infected cell cultures. DE LA TORRE et al. (1990) on the other hand, found no obvious difference in size for the largest BDV RNA species when derived from BD rat brain or the MBV cell line. Although we initially estimated a size of about 8.5 kb for the largest BDV RNA (LIPKIN et al. 1990; DE LA TORRE et al. 1990; BRIESE et al. 1992), recent results indicate that the size is closer to 10 kb



(Fig. 1) (de la Torre, unpublished). Second, VANDEWOUDE et al. (1990) reported the largest RNA as poly A^+ and as being present in equal quantities of positive and negative polarity in BD rat brain extract. In contrast, others have found the largest RNA species to be poly A^- and present predominantly in negative polarity in RNA obtained from BD rat brain or MBV cells (LIPKIN et al. 1990; DE LA TORRE et al. 1990). These discrepancies led to two different models for BDV, with some authors favoring a coronavirus model (VANDEWOUDE et al. 1990; RICHT et al. 1991) and others suggesting that BDV is a negative, single-stranded RNA virus (LIPKIN et al. 1990; DE LA TORRE et al. 1990). The isolation and characterization of RNA contained in the virion was required in order to determine the genome polarity of BDV.

2.2 BDV Contains Genomic RNA of Negative Polarity

Based on earlier observations, that infectious material can be released from BDV-infected tissue culture cells under hypertonic conditions, a method for the isolation of viral particles has been established (PAULI and LUDWIG 1985; BRIESE et al. 1992). Infectious BDV particles were released from tissue culture cells by a buffered 250 m*M* $MgCl_2$ solution and subjected to mild detergent treatment followed by extensive DNase/RNase digestion. Nucleic acid obtained from such released virus preparations revealed only a single RNA of approximately 8.5 kb when probed with the available BDV cDNAs in northern hybridization experiments (Fig. 2). Strand-specific hybridizations using RNA and oligonucleotide probes representing ORF p24 or ORF p40 detected only negative polarity RNA in released virus preparations (Fig. 2) (BRIESE et al. 1992). These results characterize BDV as a negative strand RNA virus; however, additional genomic RNAs not detected by the presently available cDNAs cannot be excluded. Thus, whether BDV has a segmented and/or ambisense genome remains to be determined.

Fig. 1A–C. Characterization of Borna disease virus (BDV) as a RNA virus.
A BDV-specific RNAs. Northern hybridization of total RNA obtained from infected (*I*) and noninfected (*N*) tissue culture cells using ^{32}P-labeled, hexamer-primed DNA probes representing either ORF p40 (p38) or ORF p24 (p24). Positions of RNA markers are indicated by their size in kb. **B** BDV sequences are not present in cellular or episomal DNA (DE LA TORRE et al. 1990). Southern hybridization of *EcoRV*-digested genomic and episomal DNA obtained from MBV and uninfected MDCK cells using a ^{32}P-labeled, hexamer-primed, internal *EcoRV* fragment from the ORF p40 representing cDNA (*top*). As control, the membrane was stripped from the BDV probe and rehybridized to a somatostatin probe (*bottom*). *Lanes 1, 3* genomic DNA (15 μg) from MDCK or MBV cells, respectively; *lanes 2, 4*, episomal DNA (7 μg) from MDCK or MBV cells, respectively; *lane 5* episomal DNA (20 μg) from MBV cells; *lanes 6,7 and 8*, *EcoRV*-digested plasmid DNA containing ORF p40 cDNA, 100, 10 and 1 pg, respectively. Positions of DNA markers are indicated by their size in kb. **C** The largest BDV RNA found in BD rat brain has the same size as the largest one found in BDV-infected tissue culture cells. Northern hybridization using a ^{32}P-labeled, hexamer-primed DNA probe representing ORF p24 and the following RNA samples: *lane 1*, RNA marker ladder (BRL #56205A) containing vesicular stomatitis virus genomic RNA (~11 kb); *lane 2*, total RNA from C6 cells; *lane 3*, nuclear, polyA⁻ RNA from MBV cells; *lane 4*, nuclear, polyA⁻ RNA from BDV infected C6 cells; *lanes 5 and 6*, total RNA from uninfected and BD rat brain, respectively. Positions of RNA markers were determined by methylene blue staining of the membrane and are indicated in *lane 1* by their size in kb

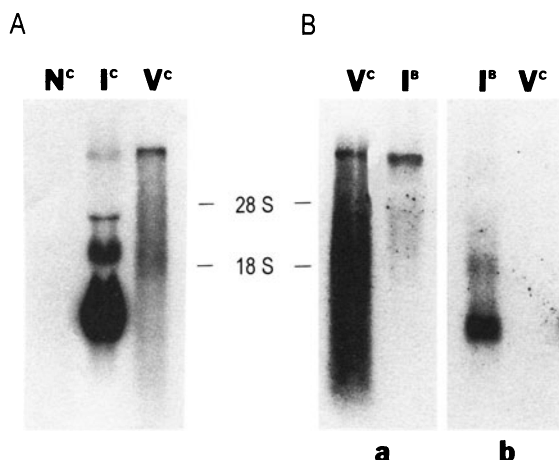


Fig. 2A, B. The largest Borna disease virus (BDV) RNA is genomic and of negative polarity.
A Released virus preparations contain the 8.5 kb BDV RNA (BRIESE et al. 1992). Northern hybridization of total RNA obtained from uninfected (N^c) and infected (I^c) tissue culture cells or released virus preparation (V^c) using a ^{32}P-labeled, hexamer-primed DNA probe representing ORF p24. **B** The 8.5 kb genomic RNA is of negative polarity (BRIESE et al. 1992). Northern hybridization of total RNA obtained from infected rat brain (I^B) or released virus preparation (V^c) using ^{32}P-labeled, single-stranded RNA probes representing either the antisense strand (*b*) or the sense strand (mRNA orientation) (*a*) of ORF p24. Positions of 18S and 28S rRNAs are indicated

The BDV-specific cDNA clones isolated so far provide a total of about 2 kb of sequence information for BDV (THIERER et al. 1992; MCCLURE et al. 1992). Reverse transcriptase PCR experiments showed that the two ORFs, ORF p40 and ORF p24, are located directly adjacent to each other on the genomic RNA. Amplification of an intervening sequence in these experiments, using a sense ORF p40 primer for reverse transcriptase in conjunction with an antisense ORF p24 primer in PCR, indicated that ORF p24 maps 105 nucleotides 5' with respect to ORF p40 on the negative strand BDV genome (Lipkin, unpublished).

3 Transcription

3.1 Transcription of BDV Occurs in the Nucleus of the Infected Cell

Two observations suggested the possibility that BDV might have a nuclear phase during transcription and/or replication. First, though the 38/40 kDa and the 24 kDa antigens are found in the cytoplasm, they are also present at high levels in the nucleus of infected cells (LUDWIG et al. 1988). Second, the production of these proteins can be prevented by actinomycin D (DUCHALA et al. 1989). More recently,

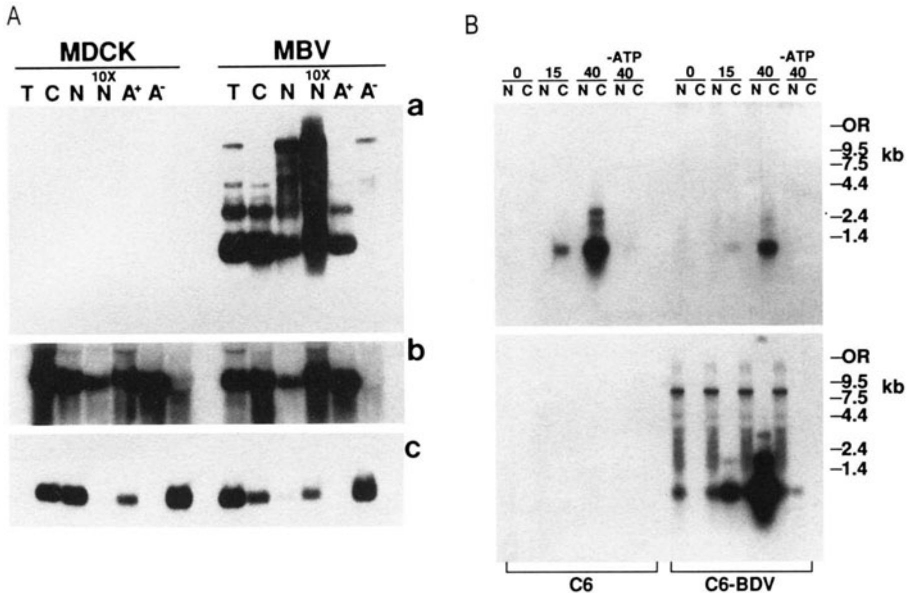


Fig. 3A, B. Poly A⁺ Borna disease virus (BDV) RNAs are transcribed in the nucleus of the infected cell. **A** Cellular distribution and polyadenylation status of BDV RNAs. Northern hybridization of RNA obtained from MDCK (*MDCK*) or MBV (*MBV*) cells after cell fractionation or polyA⁺ selection using ^{32}P-labeled, hexamer-primed DNA probes representing ORF p24 (*a*); vimentin, a polyA⁺ mRNA (*b*) or histone 2b, a non polyadenylated mRNA (*c*). T, RNA obtained from total cells; *C*, RNA obtained from cytoplasmic fraction; *N*, RNA obtained from nuclei; *A⁺*, polyA⁺ RNA; *A⁻*, polyA⁻ RNA. **B** Transcription of BDV takes place in the nucleus of the infected cell (BRIESE et al. 1992). Nuclei from C6 and BDV-infected C6 cells were isolated and RNA nucleocytoplasmic transport assays performed. At indicated time points (0, 15, 40 min) RNA was extracted from nuclear (*N*) and postnuclear (*C*) fractions and analyzed by northern hybridization using ^{32}P-labeled, hexamer-primed DNA probes representing ORF p24 (*bottom*) or the housekeeping gene cyclophilin (*top*). As a control, RNA nucleocytoplasmic transport assays were performed in the absence of ATP (–ATP, 40 min). OR, lane origin

studies of the cellular distribution of BDV RNAs after cell fractionation demonstrated that the largest, polyA⁻ BDV RNA is restricted to the nucleus, whereas the smaller, polyA⁺ RNAs are found in both the nuclear and cytoplasmic fractions (Fig. 3) (DE LA TORRE et al. 1990). The results obtained by cell fractionation were further confirmed by in situ hybridization experiments showing BDV RNAs of positive polarity in a diffuse distributed manner over the entire infected cell, whereas the genomic RNA of negative polarity was confined to the nucleus (CARBONE et al. 1991). To investigate whether transcription of BDV occurs in the nucleus of the infected cell, RNA nucleocytoplasmic transport assays were performed (BRIESE et al. 1992). Nuclei from infected tissue culture cells were isolated, resuspended in nuclear transport buffer and RNA was extracted at various time points from the nuclear and postnuclear fractions and analyzed by northern hybridization using a ORF p24 probe. As shown in Fig. 3, the 0.8, 2.1 and 3.5 kb polyA⁺ RNAs accumulated in the postnuclear fraction in a time-dependent manner. Nucleocytoplasmic transport of these RNAs was prevented

in the absence of ATP, indicating a specific, energy-dependent transport of the transcripts. These results indicate that transcription of BDV, a negative, single-stranded RNA virus, takes place in the nucleus of the infected cell (BRIESE et al. 1992).

4 Proteins

4.1 Borna Disease-Specific Proteins

Borna disease is associated with the presence of a soluble antigen (s-antigen) (VON SPROCKHOFF and NITZSCHKE 1955). The s-antigen is found in high quantities in the noninfectious supernatant obtained after high speed centrifugation of sonicated, infected brain or tissue culture cells. Diseased animals have high titers of polyclonal serum antibodies and specific oligoclonal CSF antibodies that are directed against this antigen (LUDWIG et al. 1977; LUDWIG and THEIN 1977; DANNER et al. 1978). The two major components of the s-antigen are the 38/40 kDa and the 24 kDa proteins (LUDWIG et al. 1977, 1988; LUDWIG and BECHT 1977; BAUSE-NIEDRIG et al. 1992). These proteins are not only diffusely distributed in the cytoplasm, but are also present in the nucleus of infected cells, where they appear as circumscribed aggregates (WAGNER et al. 1968; DANNER 1977; LUDWIG and BECHT 1977; BAUSE-NIEDRIG et al. 1991). Colocalization studies, together with the finding that both proteins copurify, indicate that these proteins are likely to form a high molecular weight complex in the cell (BAUSE-NIEDRIG et al. 1991, 1992).

Based on identical immunologic properties, it has been suggested that the 38 kDa and 40 kDa polypeptides are closely related and presumably represent modified forms of the same protein (HAAS et al. 1986; BAUSE-NIEDRIG et al. 1992). The 24 kDa protein, in contrast, appears to be a different, unrelated protein (see also Sect, 4.2). Though monoclonal antibodies reactive with both proteins have been isolated (THIEDEMANN et al. 1992) indicating the presence of one or more common epitopes, polyclonal sera raised against each of the polypeptides reacted only with the 24 kDa or the 38/40 kDa protein but not with both (BAUSE-NIEDRIG et al. 1992). The nature of the modification which accounts for the different M_r of the 38 and 40 kDa polypeptides is still unclear; neither phosphorylation nor glycosylation has been demonstrated for either of the 38 or 40 kDa polypeptides. The 24 kDa protein is phosphorylated (THIEDEMANN et al. 1992).

Immunoblot analysis of s-antigen preparations indicated, in addition to the 38/40 kDa and 24 kDa proteins, the presence of a 60 kDa polypeptide, predominantly found in preparations from BD rabbit brain (LUDWIG et al. 1988). While it is possible that the 60 kDa signal represents an additional, independent protein, recent data indicate that it is likely to represent a multimeric form of p24 that is rather stable towards treatment with reducing agents. Evidence for such a multimeric form of p24 comes from three observations. First, incubation of antigen preparations with

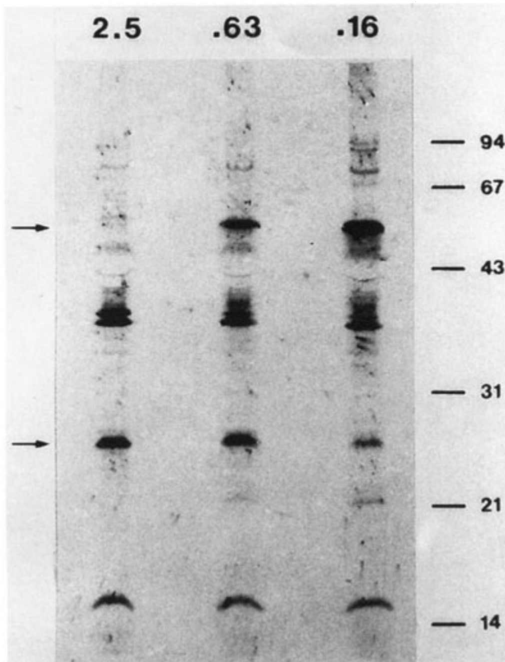


Fig. 4. Borna disease virus (BDV)-specific proteins. The 60 kDa/24 kDa signal is dependent on dithiothreitol (DTT) concentration. Immunoblot of crude protein from $MgCl_2$-released virus using anti-BDV serum and alkaline phosphatase conjugate as second antibody in conjunction with a fast red/naphthol phosphate stain. Concentrations of DTT are indicated on *top* in m*M*. *Arrows* indicate position of 60 kDa and 24 kDa signal. The position of molecular weight markers are indicated by their M_r in kDa

increasing dithiothreitol concentrations resulted in a loss of 60 kDa signal with a concomitant increase in 24 kDa signal (Fig. 4) (Briese and Ludwig, unpublished). Second, purified 24 kDa protein ran either as a 24 kDa or a 60 kDa band depending on the use of reducing or nonreducing conditions in SDS-PAGE, respectively (Briese and Ludwig, unpublished). Third, recombinant 24 kDa protein was also found in a 60 kDa position when nonreducing SDS-PAGE conditions were used (Thibault, Kliche, Briese, and Lipkin, unpublished).

Finally, a proteinase K resistant protein with a M_r of 14.5 kDa has been described (Schädler et al. 1985). The 14.5 kDa protein was isolated from brains of neonatally infected rats using a detergent/salt extraction protocol. Like the 38/40, 24 and 60 kDa antigens, it is exclusively found in infected material, but usually not in s-antigen preparations. The localization of the 14.5 kDa protein in the infected cell is not known.

4.2 Analysis of Cloned Viral Proteins

The two cDNA species cloned from BDV contain potential ORFs for a 39.5 kDa or a 22.5 kDa protein, respectively. Their deduced amino acid sequences are in agreement with partial amino acid sequence obtained through direct sequencing of either purified 40 kDa or purified 24 kDa antigen from BDV-infected material (Thierer et al. 1992; McClure et al. 1992). These data provide conclusive evidence that the cloned viral mRNAs encode two proteins, p40 and p24, which have

immunologically been characterized as the 40 kDa and the 24 kDa components of the BD-specific s-antigen. Amino acid sequence comparison reveals no significant homology between p24 and p40. In hybrid arrest experiments translation of both the 38 kDa and 40 kDa antigen is blocked by cDNA representing ORF p40 (LIPKIN et al. 1990). In bacterial expression systems ORF p40 directs translation of a product that runs as a double band with a M_r of 38/40 kDa in SDS-PAGE (Lipkin, unpublished). These results, in conjunction with the immunologic data, demonstrate a close relationship for these two polypeptides and suggest that the 38 kDa antigen represents a modified version of p40. Presently, we have no mechanism to explain how the ORF p40 directs translation of both the 38 kDa and 40 kDa polypeptides.

Database searches for similarities with other reported sequences have indicated a distant similarity of p40 to a duplicated domain of L-polymerases from paramyxo- and rhabdoviruses (MCCLURE et al. 1992). Paramyxo- and rhabdoviruses are members of the order *Mononegavirales*, enveloped RNA viruses with nonsegmented genomes of negative polarity, to which BDV may be related. However, this similarity concerns one domain of a single protein. L-polymerases are multifunctional enzymes more that 200 kDa in size and are synthesized in low abundance. In contrast, p40 is much smaller and is present in much higher amounts than would be anticipated for a functional polymerase enzyme. Nonetheless, p40 is present in the nucleus of the infected cell and might act in concert with p24, which shares the nuclear location. Colocalization studies using monoclonal antibodies against the two proteins have indicated that both proteins are present in a high molecular weight complex found in the nucleus of infected cells (BAUSE-NIEDRIG et al. 1991). In accordance with their nuclear localization, putative nuclear localization signals are found in both proteins. For p40 the positively charged sequence $K^{149}KRFK^{153}$ has been proposed as a nuclear targeting signal, whereas for p24 the basic amino acid cluster $R^{22}RKRSGSPRPRK^{33}$ has been proposed to serve this function because of its similarity to a sequence motif shown to determine the nuclear localization of hepatitis B virus core antigen (MCCLURE et al. 1992; THIERER et al. 1992).

The accumulation of high amounts of p40 and p24 in infected cells could also be compatible with a function of the proteins as structural components of BDV. Even their nuclear localization would be consistent with a structural role analogous to the nucleoprotein in the influenza virus system. If present in the virion, then p40 and p24 are unlikely to be exposed at the surface, because no labeling of BDV particles could be achieved with antibodies against p40 or p24 (Lipkin and Ribak, unpublished). Further biochemical studies will be needed to assign any function to these proteins.

5 Concluding Remarks

Though the body of data accumulated over the past 3 years has led to consensus on the characterization of BDV as a RNA virus, some aspects of BDV's biology are still controversial.

VandeWoude, Richt and coworkers had suggested that the BD-specific 14.5 kDa protein is a virus-encoded protein expressed from a smaller ORF present within the cDNA clone containing ORF p24 that overlaps with ORF p24 (Vande Woude et al. 1990; Richt et al. 1991). However, partial amino acid sequence from BD-specific 14.5 kDa protein purified following the method of Schädler et al. (1985) (NSKHSYV; Kliche et al. 1994) does not match the amino acid sequence deduced from the ORF proposed by Richt, VandeWoude and coworkers (Richt et al. 1991). Instead, the amino acid data identified a potential ORF p16, residing adjacent to the p24 gene in nucleic acid sequence obtained from genomic BDV cDNA clones. This suggests that the 14.5 kDa protein is a viral protein encoded by a gene separate from ORF p24 (Kliche et al. 1994).

Table 1. Characterization of Borna disease virus

Nucleic acids	
Negative, single-stranded genomic RNA	
	8.5–10.5 kb
poly (A)$^+$ transcripts transcribed in the nucleus	
	0.8 kb p24 mRNA
	1.2 kb p40/38 mRNA
	2.1 kb (precursor, 1.2 kb + 0.8 kb)?
	3.5 kb (precursor, 0.8 kb + ?)?
Proteins	
38/40 kDa protein	
	Virus-encoded
	Component of s-antigen (complex)
	Cytoplasmic and nuclear localization
	Putative nuclear localization signal
	Distant similarity to polymerases of paramyxo- and rhabdoviruses
	Function unknown
24 kDa protein	
	Virus-encoded
	Component of s-antigen (complex)
	Cytoplasmic and nuclear localization
	Putative nuclear localization signal
	Ability to form 60 kDa multimer (dimer?)
	Function unknown
14 kDa protein	
	Virus-encoded
	Proteinase K resistant
	Function unknown
60 kDa antigen	
	Component of s-antigen (complex)
	(Multimeric form of p24?)
	Function unknown
Morphology	
(Enveloped, spherical 90 nm particle)	

In the same publications, RICHT, VANDEWOUDE and coworkers have discussed the occurrence of a nested set of overlapping subgenomic, positive- and negative-strand RNAs in BDV, proposing a similarity of BDV to coronaviruses, which synthesize nested sets of overlapping subgenomic mRNAs that are 3'-coterminal with the genome (VANDEWOUDE et al. 1990; RICHT et al. 1991). However, Fig. 1 shows that BDV synthesizes several transcripts that are not coterminal, which argues against a coronavirus model.

Table 1 summarizes the present knowledge of the molecular biology of BDV. BDV is a single-stranded RNA virus containing a genomic RNA of 8.5–10.5 kb. This genomic RNA is of negative polarity for sequences complementary to the two mRNAs presently cloned from BDV; no data are available to decide whether the BDV genome is segmented and/or ambisense. Four polyA$^+$ transcripts of 3.5, 2.1,1.2 and 0.8 kb are identified by the available BDV cDNAs. Transcription of the RNAs takes place in the nucleus as determined by RNA nucleocytoplasmic transport assays. The role of the 2.1 and 3.5 kb BDV transcripts is unclear. Based on its size, the 2.1 kb RNA could represent a dicistronic RNA of ORF p40 plus ORF p24; the 3.5 kb RNA might contain other, additional gene(s). These RNAs may represent read-through artifacts or function as precursor molecules which are processed into individual mRNAs. BDV mRNAs of 1.2 kb and 0.8 kb direct the translation of p40 and p24, respectively, two viral proteins that have previously been characterized as the major components of the BD-specific s-antigen. Both proteins are found in the cytosol and in the nucleus of infected cells and have motifs consistent with nuclear localization signals. Comparative sequence analysis has revealed a distant similarity of p40 to L-polymerases of paramyxo- and rhabdoviruses. In addition to p40 and p24, BDV also encodes p16, a protein described as the BD-specific 14.5 kDa protein.

BDV still remains unclassified. Among RNA viruses only orthomyxoviruses, which have a negative-stranded, segmented genome, are known to transcribe in the nucleus of the infected cell (HERZ et al. 1981; JACKSON et al. 1982). Thus, if a nonsegmented genome is confirmed, BDV might be considered to represent a new class of virus, and it would be conceivable that other BDV related viruses will be found.

Identification of a new BDV-related class of viruses might have potential implications for human disease: immunologic data indicate that BDV/BDV-related viruses may infect humans (see Bode, this volume), and some manifestations of BD resemble human neuropsychiatric disorders of unknown cause (see Solbrig, Fallon and Lipkin, this volume). Studies into the molecular genetics of BDV now provide sensitive probes to evaluate the possible involvement of BDV/BDV-related viruses in human CNS disorders. Evidence that viruses can induce progressive neurological disorders has encouraged studies to understand the mechanisms and biologic consequences of persistent virus infection of the CNS (GILDEN and LIPTON 1989; TER MEULEN 1991). In this regard, BDV has become an attractive model for the investigation, not only of immune-mediated pathological events in virally induced neurological disease (Stitz, Dietzschold and Carbone, this volume), but also of the mechanisms by which virus infection of the CNS can lead to subtle disturbances in behavior (SPRANKEL et al. 1978; DITTRICH et al. 1989).

Further, understanding of the molecular genetics and biology of BDV offers the opportunity to characterize the basis for its unique tropism. The unique aspects of BDV tropism anticipate its importance for neurobiology and clinical medicine. Studies are already underway to use BDV as a tool for mapping connectivity within the nervous system. As the molecular basis for pathogenicity becomes clear, it may be feasible to establish BDV-based vectors for targeted drug delivery within the brain.

Acknowledgements: Support for this work was provided by NIH grants NS-29425 (T.B., W.I.L.); NS-12428 (J.C.T.); UC Taskforce on AIDS-R911047 (T.B.,W.I.L.). T.B. is a recipient of a Young Investigator Award from NARSAD. W.I.L. is recipient of a Young Investigator Award from NARSAD and a Pew Scholars Award from the Pew Charitable Trusts.

References

Amsterdam JD, Winokur A, Dyson W, Herzog S, Gonzalez F, Rott R, Koprowski H (1985) Borna disease virus. A possible etiologic factor in human affective disorders? Arch Gen Psychiat 42: 1093–1096

Anzil AP, Blinzinger K, Mayr A (1973) Persistent Borna virus infection in adult hamsters. Arch Gesamte Virusforsch 40: 52–57

Bause-Niedrig I, Pauli G, Ludwig H (1991) Borna disease virus-specific antigens: two different proteins identified by monoclonal antibodies. Vet Immunol Immunopathol 27: 293–301

Bause-Niedrig I, Jackson M, Schein E, Ludwig H, Pauli G (1992) Borna disease virus-specific antigens. II. The solube antigen is a protein complex. Vet Immunol Immunopathol 31: 361–369

Bode L, Riegel S, Ludwig H, Amsterdam JD, Lange W, Koprowski H (1988) Borna disease virus-specific antibodies in patients with HIV infection and with mental disorders. Lancet ii: 689

Bode L, Riegel S, Lange W, Ludwig H (1992) Human infections with Borna disease virus: seroprevalence in patients with chronic diseases and healthy individuals. J Med Virol 36: 309–315

Briese T, de la Torre JC, Lewis A, Ludwig H, Lipkin WI (1992) Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. Proc. Natl. Acad. Sci. USA 89: 11486–11489

Briese T, Schneemann A, Lewis AJ, Park YS, Kim S, Ludwig H, Lipkin WI (1994) Genomic organization of Borna disease virus. Proc Natl Acad Sci USA 91: 4362–4366

Carbone KM, Duchala CS, Griffin JW, Kincaid AL, Narayan O (1987) Pathogenesis of Borna disease in rats: evidence that intra-axonal spread is the major route for virus dissemination and the determinant for disease incubation. J. Virol. 61: 3431–3440

Carbone KM, Moench TR, Lipkin WI (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

Cubitt B, Oldstone C, de la Torre JC (1994) Sequence and genome organization of Borna disease virus. J Virol 68: 1382–1396

Cubitt B, Oldstone C, Valcarel V, de la Torre JC (1994) RNA splicing contributes to the generation of mature mRNAs of Borna disease virus, a non-segmented negative strand RNA virus. Virus Res 34: 69–79

Cubitt B, de la Torre JC (1994) Borna disease virus (BDV), a nonsegmented RNA virus, replicates in nuclei of infected cells where infectious BDV ribonucleoproteins are present. J Virol 68: 1371–1381

Compans RW, Melsen LR, de la Torre JC (1994) Virus-like particles in MDCK cells persistently infected with Borna disease virus. Virus Res 33: 261–268

Danner K (1977) Borna disease: patterns of infection. In: ter Meulen V, Katz M (eds) Slow virus infections of the central nervous system. Springer, Berlin Heidelberg New York, pp 84–88

Danner K, Mayr A (1979) *In vitro* studies on borna virus. II. Properties of the virus. Arch. Virol. 61: 261–271

Danner K, Lüthgen K, Herlyn M, Mayr A (1978) Vergleichende Untersuchungen über Nachweis und Bildung von Serumantikörpern gegen das Borna-Virus (in German). Zentralbl Vet Med [B] 25: 345–355

de la Torre JC, Carbone KM, Lipkin WI (1990) Molecular characterization of the borna disease agent. Virology 179: 853–856

Dittrich W, Bode L, Ludwig H, Kao M, Schneider K (1989) Learning deficiencies in Borna disease virus-infected but clinically healthy rats. Biol Psychiatry 26: 818–828

Duchala CS, Carbone KM, Narayan O (1989) Preliminary studies on the biology of Borna disease virus. J Gen Virol 70: 3507–3511

Elford WJ, Galloway IA (1933) Filtration of the virus of Borna disease through graded collodion membranes. Br J Exp Pathol 14: 196–205

Gilden DH, Lipton HL (eds) (1989). Clinical and molecular aspects of neurotropic virus infection. Kluwer Academic, Boston

Gosztonyi G, Ludwig H (1984) Borna disease of horses. An immunohistological and virological study of naturally infected animals. Acta Neuropathol (Berl) 64: 213–221

Haas B, Becht H, Rott R (1986) Purification and properties of an intranuclear virus-specific antigen from tissue infected with borna disease virus. J Gen Virol 67: 235–241

Heinig A (1969) Die Bornasche Krankheit der Pferde und Schafe (in German). In: Röhrer H (ed) Handbuch der Virusinfektionen bei Tieren, Vol 4. Fischer, Jena, pp 83–148.

Herz C,Stavnezer E, Krug R, Gurney T (1981) Influenza virus, an RNA virus, synthesizes its messenger RNA in the nucleus of infected cells. Cell 26: 391–400

Hirano N, Kao M, Ludwig H (1983) Persistent, tolerant or subacute infection in Borna disease virus-infected rats. J Gen Virol 64: 1521–1530

Jackson DA, Caton AJ, McCready SJ, Cook PR (1982) Influenza virus RNA is synthesized at fixed sites in the nucleus. Nature 296: 366–368

Joest E, Degen K (1911) Untersuchungen über die pathologische Histologie, Pathogenese und postmortale Diagnose der seuchenhaften Gehirn-Rückenmarksentzündung (Bornasche Krankheit) des Pferdes (in German). Z Inf Krkh Haustiere 9: 1–98

Kao M, Ludwig H, Gosztonyi G (1984) Adapation of borna disease virus to the mouse. J Gen Virol 65: 1845–1849

Kliche S, Briese T, Henschen AH, Stitz L, Lipkin WI (1994) Characterization of a Borna disease virus glycoprotein, gp18. J Virol 68: 6918–6923

Krey H, Ludwig H, Rott R (1979) Spread of infectious virus along the optic nerve into the retina in Borna disease virus-infected rabbits. Arch Virol 61: 283–288

Lipkin WI, Travis GH, Carbone KM, Wilson MC (1990) Isolation and characterization of Borna disease agent cDNA clones. Proc Natl Acad Sci USA 87: 4184–4188

Ludwig H, Becht H (1977) Borna, the disease—a summary of our present knowledge. In: ter Meulen V, Katz M (eds) Slow virus infections of the central nervous system. Springer, Berlin Heidelberg New York, pp 75–83

Ludwig H, Thein P (1977) Demonstration of specific antibodies in the central nervous system of horses naturally infected with Borna disease virus. Med Microbiol Immunol 163: 215–226

Ludwig H, Becht H, Groh L (1973) Borna disease (BD), a slow virus infection. Biological properties of the virus. Med Microbiol Immunol 158: 275–289

Ludwig H, Koester V, Pauli G, Rott R (1977) The cerebrospinal fluid of rabbits infected with borna disease virus. Arch Virol 55: 209–223

Ludwig H, Kraft W, Kao M, Gosztonyi G, Dahme E, Krey H (1985) Borna-Virus-Infektion (Borna-Krankheit) bei natürlich und experimentell infizierten Tieren: ihre Bedeutung für Forschung und Praxis (in German). Tierarztl Prax 13: 421–453

Ludwig H, Bode L, Gosztonyi G (1988) Borna disease: a persistent virus infection of the central nervous system. Prog Med Virol 35: 107–151

Matthias D (1954) Der Nachweis von latent infizierten Pferden, Schafen und Rindern und deren Bedeutung als Virusreservoir bei der Bornaschen Krankheit (in German). Arch Exp Vet Med 8: 506–511

McClure MA, Thibault KJ, Hatalski CG, Lipkin WI (1992) Sequence similarity between Borna disease virus p40 and a duplicated domain within the paramyxovirus and rhabdovirus polymerase proteins. J Virol 66: 6572–6577

Metzler A, Frei U, Danner K (1976) Virologisch gesicherter Ausbruch der Bornashen Krankheit in einer Schafherde der Schweiz (in German). Schweiz Arch Tierheilkd 118: 483–492

Morales JA, Herzog S, Kompter C, Frese K, Rott R (1988) Axonal transport of Borna disease virus along olfactory pathways in spontaneously and experimentally infected rats. Med Microbiol Immunol 177: 51–68

Narayan O, Herzog S, Frese K, Scheefers H, Rott R (1983) Pathogenesis of Borna disease in rats: immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. J Infect Dis 148: 305–315

Nicolau S, Galloway IA (1928) Borna disease and enzootic encephalo-myelitis of sheep and cattle. Stationary Office, London (Privy Council, Medical Research Council, special report series no 121)

Nitzschke E (1963) Untersuchungen über die experimentelle Bornavirus-Infektion bei der Ratte (in German). Zentralbl Vet Med [B] 10: 470–527
Pauli G, Ludwig H (1985) Increase of virus yields and releases of Borna disease virus from persistently infected cells. Virus Res. 2: 29–33.
Pauli G, Grunmach J, Ludwig H (1984) Focus-immunoassay for borna disease virus-specific antigens. Zentralbl Vet Med [B] 31: 552–557
Richt JA, VandeWoude S, Zink MC, Narayan O, Clements JE (1991) Analysis of Borna disease virus-specific RNAs in infected cells and tissues. J Gen Virol 72: 2251–2255
Richt JA, VandeWoude S, Zink MC, Clements JE, Herzog S, Stitz L, Rott R, Narayan O (1992) Infection with Borna disease virus: molecular and immunobiological characterization of the agent. Clin. Infect Dis 14: 1240–1250
Rott R, Herzog S, Fleischer B, Winokur A, Amsterdam J, Dyson W, Koprowski H (1985) Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. Science 228: 755–756
Rott R, Herzog S, Bechter K, Frese K (1991) Borna disease, a possible hazard for man? Arch Virol 118: 143–149
Schädler R, Diringer H, Ludwig H (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
Schneeman A, Schneider PA, Kim S, Lipkin WI (1994) Identification od signal sequences that cintrol transcription of Borna disease virus, a nonsegmented, negative-strand RNA virus. J Virol 68: 6514–6522
Schneider S, Breise T, Henschen AH, Stitz L, Lipkin WI (1994) Characterization of a Borna disease virus glycoprotein, gp18. J Virol 68: 6918–6923
Seifried O, Spatz H (1930) Die Ausbreitung der encephalitischen Reaktion bei der Bornaschen Krankheit der Pferde und deren Beziehungen zu der Encephalitis epidemica, der Heine-Medinschen Krankheit und der Lyssa des Menschen. Eine vergleichend-pathologische Studie (in German). Z Gesamte Neurol Psychiatry 124: 317–382
Sprankel H, Richarz K, Ludwig H, Rott R (1978) Behavior alterations in tree shrews (Tupaia glis, Diard 1820) induced by Borna diseasae virus. Med Microbiol Immunol 165: 1–18
Stitz L, Krey H, Ludwig H (1980) Borna disease in Rhesus monkeys as a model for uveo-cerebral symptoms. J Med Virol 6: 333–340
ter Meulen V (ed) (1991) Virus-cell interactions in the nervous system. Seminars in neuroscience, Vol 3. London, Saunders, pp 81–123
Thiedemann N, Presek P, Rott R, Stitz L (1992) Antigenic relationship and further characterization of two major Borna disease virus-specific proteins. J Gen Virol 73: 1057–1064
Thierer J, Riehle H, Grebenstein O, Binz T, Herzog S, Thiedemann N, Stitz L, Rott R, Lottspeich F, Niemann H (1992) The 24K protein of Borna disease virus. J Gen Virol 73: 413–416.
VandeWoude S, Richt JA, Zink MC, Rott R, Narayan O, Clements JE (1990) A Borna virus cDNA encoding a protein recognized by antibodies in humans with behavioral diseases. Science 250: 1278–1281
von Sprockhoff H (1956) Zur biologischen Charakterisierung des Borna-s-Antigens (in German). Z. Immun Forsch (Jena) 113: 379–385
von Sprockhoff H, Nitzschke E (1955) Untersuchungen über das komplementbindende Antigen in Gehirnen bornavirus-infizierter Kaninchen. 1. Mitteilung: Nachweis eines löslichen Antigens (in German). Zentralbl Vet Med 2: 185–192
Waelchli RO, Ehrensperger F, Metzler A, Winder C (1985) Borna disease in a sheep. Vet Rec 117: 499–500
Wagner K, Ludwig H, Paulsen J (1968) Fluoreszenzserologischer Nachweis von Borna-Virus Antigen (in German). Berl Munch Tierarztl Wochenschr 81: 395–396
Zimmermann W, Breter H, Rudolph M, Ludwig H (1994) Borna disease virus: immunoelectron microscopic characterization of cell-free virus and further information about the genome. J Virol 68: 6755–6758
Zwick W (1939) Bornasche Krankheit und Enzephalomyelitis der Tiere (in German). In: Gildemeister E, Haagen E, Waldmann O (eds) Handbuch der Viruskrankheiten, vol 2. Fischer, Jena, pp 254–354
Zwick W, Seifried O, Witte J (1926) Experimentelle Untersuchungen über die seuchenhafte Gehirn- und Rückenmarksentzündung der Pferde (Bornasche Krankheit) (in German). Z Inf Krankh Haustiere 30: 42–136

Note Added in Proof

Since this chapter was written, the BDV genome has been cloned and sequenced, subgenomic RNAs have been mapped to the viral genome and viral particles have been identified by immunoelectron microscopy. BDV has a nonsegment 8.9 kb genome with complementary 3' and 5' termini that contains antisense information of five ORFs (BRIESE et al. 1994; CUBITT and DE LA TORRE 1994). Unlike genomes of other nonsegmented, negative strand RNA viruses, the BDV genome does not have distinct gene boundaries. Instead, transcription units and transcription signals frequently overlap (SCHNEEMANN et al. 1994). Subgenomic RNAs encoding p40 and p24 are monocistronic. In contrast, RNAs encoding the putative matrix, glycoprotein and polymerase proteins are polycistronic and undergo posttranscriptional modification by RNA splicing (BRIESE et al. 1994; CUBITT et al. 1994; SCHNEEMANN et al. 1994; SCHNEIDER et al. 1994). Morphologically the virion appears to be a 90 nm enveloped, spherical particle containing an electron dense internal structure consisting of strand-like material (ZIMMERMANN et al. 1994; COMPANS et al. 1994). In concert, these features indicate that BDV represents the prototype of a new group of animal RNA viruses within the order *Mononegavirales.*