Bornavirus and the Brain

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Borna disease virus (BDV) causes central nervous system (CNS) disease that is frequently manifested by behavioral abnormalities. BDV is a nonsegmented, negative, single-stranded RNA virus. On the basis of its unique genetic and biologic features, BDV is the prototypic member of a new virus family, Bornaviridae, within the order Mononegavirales. Therefore, the investigation of the molecular and cell biology of BDV may provide new insights about virus-cell interactions in the CNS. BDV is an important model system for the investigation of viral persistence in the CNS. Serologic and molecular epidemiologic studies suggest that BDV can infect humans. Despite controversy about potential association with human neuropsychiatric illnesses, BDV affords an intriguing model for the study of these illnesses. Neonatal BDV-infected rats display neurodevelopmental, physiologic, and neurobehavioral abnormalities that closely parallel some of the main features associated with several human mental disorders.

Persistent virus infections in the central nervous system (CNS) can induce progressive neurologic disorders associated with diverse pathologic manifestations [1, 2]. These findings have led to the hypothesis that viruses can contribute to human mental diseases with elusive etiology. The consequences of viral infections of the CNS are determined by complex interaction between the virus and its host. Thus, the genetics, age, and immune status of the host as well as viral tropism for selected populations of brain cells can significantly influence the outcome of the infection. To establish a persistent infection a virus must avoid clearance by the host immune surveillance and adopt a strategy of multiplication compatible with host survival. To achieve these goals, viruses use a plethora of strategies [3, 4]. The existence of the blood-brain barrier and paucity of immune elements in the brain favor viral persistence in the CNS [5].

Borna disease virus (BDV), a nonsegmented, negative-strand (NNS) RNA virus [6, 7], causes CNS disease in a wide range of vertebrate species [8]. BDV provides an important model system for the investigation of immune-mediated pathologic events involved in virally induced neurologic disease and the mechanisms whereby viruses induce behavioral and neurodevelopmental abnormalities in the absence of overt CNS inflammation. Serologic and molecular epidemiologic evidence suggest that BDV could also infect humans and may be associated with certain neuropsychiatric disorders [9, 10]. BDV is the prototypic member of a new virus family, Bornaviridae within the order Mononegavirales [7, 11]. Consequently, investigations on BDV may provide new insights into virus-cell interactions in the CNS.

Here I discuss the present knowledge of BDV persistence in the CNS. I first describe the biology of BDV with special focus on its molecular biology, then examine the interaction between BDV and the CNS, and briefly describe the resemblance of BDV-induced functional and neuropathologic abnormalities to neuropsychiatric disorders.

The Infectious Agent

Biologic characteristics. By the late 1800s, Borna disease (BD) was recognized as a sporadically occurring encephalopathy that affected horses and sheep in central Europe [8]. Since then, BDV has been found to cause CNS disease in a broad range of vertebrate species and has become an important model for the study of CNS viral persistence [12].

Electron microscopy studies show that virions are of spherical morphology with a diameter of 70–130 nm [13, 14]. These particles contain an internal electron-dense core (50–60 nm) and a limiting outer membrane envelope covered with spikes ~7.0 nm long [13]. BDV is characterized molecularly as an NNS RNA virus, but on the basis of its unique genetic and biologic features, it is the prototypic member of the Bornaviridae family [7, 11].

BDV naturally occurring infections were thought to affect only horses and sheep in certain central Europe regions. In these species BDV can cause BD, an often-fatal immune-mediated neurologic disease [8]. More recent evidence indicates that the natural host range of BDV as well as its geographic distribution and prevalence may have been underestimated [15]. Intranasal infection is likely an important route of BDV natural infection, allowing virus access to the limbic system in the CNS by intraaxonal migration through the olfactory nerve [8]. BDV RNA and infectivity have been detected in bodily secretions

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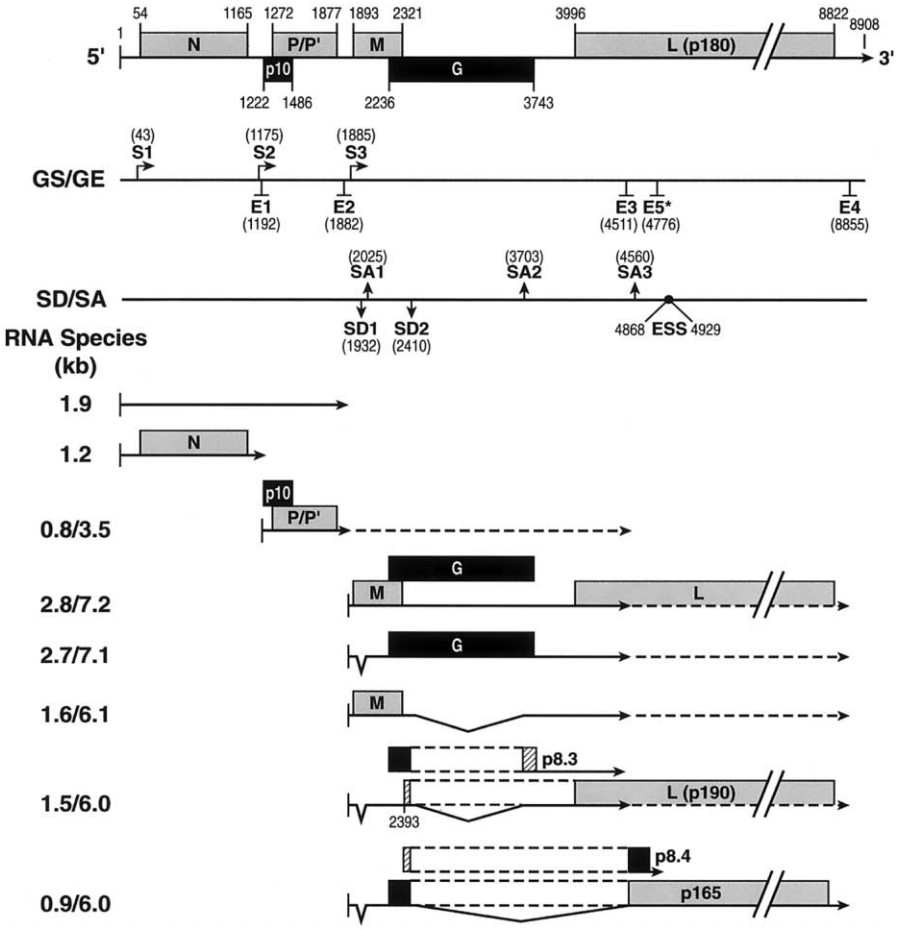

Figure 1. Genomic organization and transcriptional map of Borna disease virus (BDV). Boxes at top, BDV open-reading frames. Different shades correspond to different reading frames within the antigenomic polarity of the BDV genomic RNA. Locations of transcription initiation and transcription termination sites are indicated by S and E, respectively. Positions of BDV introns I and II are indicated.

and excretions. Thus, animals may become infected by contact with saliva, excretions, and nasal secretion [16]. The frequency of BD cases is seasonal, suggesting that environmental factors can influence the circulation of BDV in the field.

The identification of natural reservoirs of BDV should aid in understanding its intriguing epidemiology. Experimentally, BDV has a wide host range from birds to nonhuman primates and both host and viral factors contribute to a variable period of incubation and significant heterogeneity in the symptoms and pathology associated with BDV infection [8]. Immune-mediated neuronal damage is responsible for the clinical symptoms of classic BD [17]. In contrast, the mechanisms whereby BDV causes CNS disturbances in the absence of encephalitis remain largely unknown. Different BDV isolates can exhibit significant diversity in their phenotypic expression, but they usually show a remarkable sequence conservation [15]. BDV is noncytolytic and highly neurotropic, but astrocytes and to lesser extent oligodendrocytes and ependymal cells can also be infected [18].

Genome organization and encoded proteins. The BDV genome (~8.9 kb) has an organization characteristic of mononegaviruses [6, 7]. Six major open-reading frames (ORFs) are found in the BDV genome sequence (figure 1). These ORFs code for polypeptides with predicted M_r of 40 (p40), 24 (p24), 10 (p10), 16 (p16), 56 (p56), and 180 (p180) kDa, respectively. Based on their positions in the viral genome and abundance in infected cells and virion particles, together with their biochemical and sequence features, p40, p24, and p16 BDV polypeptides correspond to the viral nucleoprotein (N), the phosphoprotein (P) transcriptional activator, and matrix (M) proteins, respectively, found in other NNS RNA.

BDV ORF p56, the putative virus surface glycoprotein (G), overlaps, in a different frame, with the C-terminus of ORF p16 and is capable of encoding a 503-amino acid (aa) polypeptide with a predicted molecular mass of 56 kDa. The p56 gene directs the synthesis of 2 glycosylated polypeptides of ~84–94 kDa (gp84/94) and 43 kDa (gp43), corresponding to the full length and C-terminus, respectively, of the p56 gene. gp43 is

produced via cleavage of gp84/94 by the cellular protease furin and is the only BDV polypeptide detected at the cell surface. BDV ORF p180 is capable of encoding a polypetide whose deduced aa sequence displays homology to other NNS RNA virus polymerases, members of the L protein family.

The BDV Life Cycle

BDV cell entry is by receptor-mediated endocytosis [19]. Antibodies to G have neutralizing activity, suggesting that G is implicated in BDV adsorption, entry, or both [20]. Recent evidence indicates that the N-terminal part (aa 1–244) of BDV p56 is sufficient for receptor recognition and virus entry [21]. The N-terminus of gp43 contains a highly hydrophobic domain, and BDV-infected cells form extensive syncytia upon low pH treatment, suggesting that gp43 is involved in pH-dependent fusion after internalization of BDV by receptor-mediated endocytosis [19, 21]. This fusion event releases the BDV ribonucleoprotein (RNP), which is then transported to the cell nucleus where, unique among known animal mononegaviruses, BDV transcription and replication occur. Sequential and polar transcription results in decreasing molar quantity of BDV transcripts from the 3′- to the 5′-encoded cistrons. The viral mRNAs are polyadenylated and contain a 5′ cap structure.

The BDV genome contains 3 transcription initiation sites (S signals) and 4 transcription termination/polyadenylation sites (E signals) (figure 1). The S signals contain a semiconserved U-rich motif that is not found within the S signals of previously described NNS RNA viruses [22], whereas BDV E signals resemble the E signal motif found in other NNS RNA viruses. The BDV genome lacks the characteristic configuration of E signal/intergenic region/S signal found at the gene boundaries of other mononegaviruses. Instead, BDV transcription units and transcriptive signals frequently overlap [6, 7]. Two of the BDV primary transcripts are posttranscriptionally processed by the cellular RNA splicing machinery [6, 7].

Three introns (I, II, III) have been identified in the BDV genome (figure 1). Splicing of intron I places the aa in position 13 of M next to a stop codon, whereas splicing of intron II and I + II results in mRNAs containing 2 new ORFs. One ORF predicts a polypeptide that corresponds to the first 58 aa of G fused to a new C terminus of 20 aa, whereas the other ORF predicts a protein corresponding to a variant BDV L protein (p190) with 153 aa added to the N terminus. Intron III is generated by alternative 3′ splice site choice and covers nucleotides 2410–4559 of the BDV genome [23, 24]. Alternative splicing of introns II and III is regulated by the use of an alternative transcription termination/polyadenylation signal (GE6) and a *cis*-acting exon-splicing suppressor element located within the L gene [24]. Intron III spliced mRNAs have coding capability for 2 new viral proteins with predicted molecular masses of 8.4 and 165 (p165) kDa. p165 is a truncated form of the BDV L polymerase containing at its N-terminus the BDV G signal peptide. The potential of p165 for being secreted and the presence of 3 RGD could provide p165 with the ability to interact with integrin molecules present at the cell surface. A similar situation has been proposed for the cytomegalovirus DNA polymerase accessory protein ppM44 [25].

Virus-derived BDV mRNAs are spliced with significantly lower efficiency compared with the same plasmid-derived mRNAs [23, 24, 26]. This could prevent BDV-induced disturbances in the regulation of the cellular RNA processing machinery, thus facilitating viral persistence without compromising cell viability. In addition to increasing the virus proteomic complexity, RNA splicing can modulate the efficiency of termination-reinitiation of translation and leaky scanning mechanisms, thus contributing to the regulation of viral gene expression.

BDV polypetides, with the exception of N, are translated from polycistronic mRNAs, a unique situation among known mononegaviruses. Experimental evidence indicates that a leaky ribosome scanning mechanism contributes to the expression of the downstream ORFs [27, 28]. Completion of the BDV lifecycle requires a variety of nucleocytoplasmic transport events involving viral macromolecules. Each process is distinct, and the same viral components can be part of multiple transfer events in and out of the nucleus, each of which may utilize different mechanisms and different signals [29].

The mechanisms involved in the control of trafficking of viral RNP across the nuclear envelope and BDV mRNA nuclear export remain largely unknown. In contrast, several of the signals and interactions involved in nucleocytoplasmic transport of BDV proteins have been elucidated during the last few years. Nuclear localization signals (NLS) for NP were mapped to the 13 N-terminal aa residues, with the motif $_4$KRR$_6$ playing an essential role [30]. Consequently, the isoform p38 of N lacks this NLS and therefore accumulates in the cytoplasm. The biologic implication of the existence of 2 N isoforms with different subcellular targeting properties remains to be determined. P has 2 functionally independent proline-rich NLS located at its N- and C-terminal regions [31]. Nuclear import of p10 occurs through a nonconventional NLS [32]. The p10 sequence also contains a characteristic leucine-rich nuclear export sequence [33]. However, whether p10 has a nuclear export activity in BDV-infected cells remains to be determined.

Assembly and release of BDV are poorly understood. Thin sections of BDV-infected cells reveal the presence of intracytoplasmic virus-like particles with morphologic characteristics similar to those described for partially purified cell-free BDV infectious particles [34]. These particles show no association with cisternae of the endoplasmic reticulum, the Golgi complex, or other intracytoplasmic membranes. The assembly process and site of virus maturation have not been identified. Budding of BDV particles is only observed at the cell surface of BDV-infected MDCK cells after treatment with *n*-butyrate [13].

Whether this reflects the natural pathway for the exit of BDV remains to be determined.

BDV and the Brain

Neuroinvasion and Propagation of BDV

Evidence suggests that a primary route of BDV infection may be through the nose and the olfactory neuroepithelium [8]. After initial replication in the neurons located at the site of entry, BDV migrates intraaxonally in an anterograde or retrograde direction toward the CNS [8, 35, 36]. After intranasal inoculation of the rat, BDV initially replicates in the neuroreceptor cells of the olfactory epithelium. The virus can then gain access to the cells of the main olfactory bulb (MOB) by anterograde transport, subsequently reaching the MOB efferent targets. Further spread is then possible into numerous diencephalic and telencephalic areas through polysynaptic neuronal connections. BDV spreads transsynaptically along neuronal chains and exhibits a preferential tropism for the limbic system [36].

Virus spread is not restricted to the limbic system and BDV will diffuse throughout the CNS. In the nuclei of infected neurons, aggregates of virus-specific material form the Joest-Degen inclusion bodies characteristic of BDV infection [36]. Mature virus particles are not detected during BDV propagation within the CNS, suggesting that viral spread might ensue in the form of RNP. BDV infection affects specific neurotransmitter pathways, and its distribution in the hippocampus coincides with that of some excitatory aa receptors (e.g., glutamate). Later in infection, viral antigen and RNA can be detected in astrocytes, oligodendrocytes, ependymal cells, and Schwann cells in the peripheral nervous system. In the late stages of infection, BDV diffuses centrifugally, probably by using an anterograde axonal transport, and virus markers can be detected in peripheral nerves of all tissues and organs. Neutralizing antibodies can appear late during infection and have been implicated in restricting viral replication to the nervous system [37].

Neuropathogenesis of Classic BD

BD is defined as a nonpurulent polioencephalomyelitis caused by a T cell–dependent immune mechanism. The rat is the most common experimental model for the study of BDV pathogenesis [17, 36]. Infected adult rats develop an immune-mediated biphasic behavioral disease. Heightened viral gene expression in limbic system structures, together with astrocytosis and neuronal structural alterations within the hippocampal formation, are main histopathologic hallmarks of BDV infection. The onset of clinical signs coincides with the appearance of an inflammatory reaction in the brain that reaches its maximum of severity 30–40 days after infection. This extensive inflammatory reaction leads to neuronal destruction that in some cases may cause a hydrocephalus. Both CD4 and CD8 T cells are present in the CNS cell infiltrates and their elimination or functional blocking prevents both BDV-induced neurologic symptoms and histopathologic changes in the brain [38], suggesting that these cells contribute to the immune-mediated pathology associated with BD.

Lymphocytes isolated from rat brains during acute BD exhibit major histocompatibility complex (MHC) class I–restricted cytotoxic T lymphocyte (CTL), and expression of MHC I antigens is readily detected in astrocytes and neurons in BDV-infected rat brains [38]. Lack of MHC class I expression is thought to allow virally infected neurons to avoid destruction by CTL. Nevertheless, inhibition of electric activity and exposure to specific cytokines can induce MHC class I expression in neurons [39].

Pharmacologic studies indicate that behavioral disturbances associated with BD might be due to BDV-induced alterations of the dopamine system [40]. Decreased numbers of D2 and D3 receptor binding sites are found in the nucleus accumbens of rats with BD. Abnormalities in the mesocorticolimbic dopaminergic network may constitute the neural substrate of hyperactivity in BD. Besides dopamine, alterations in other neurotransmitter systems have been described in rats with BD, including changes in mRNA levels of cholecystokinin, glutamic acid decarboxylase, and somatostatin [41]. These pharmacologic abnormalities are likely a consequence of the inflammatory response. However, cortical cholinergic denervation was observed in BDV-infected rats in the absence of any significant cell destruction [42].

Increased levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1α, and inducible nitric oxide synthase mRNAs in BDV-infected rat brains correlate with the degree of inflammation and severity of neurologic signs [17]. Apathy, somnolence, and depression follow the initial aggressive hyperactive behavior. This chronic phase is characterized by a steady decline in the inflammatory reaction despite continuous high virus load in the CNS. A switch from a Th1-like immune cellular response to a Th2-like immune humoral response likely contributes to this delayed immune tolerance [43].

Neonatal BDV Infection of the Rat

Neurodevelopmental abnormalities. BDV neonatal infection of the rat causes a life-long persistent infection that is characterized by the lack of any significant inflammatory cell infiltration within the CNS and the absence of clinical signs of BD. This infection has been designated a "persistent, tolerant infection of the newborn" (PTI-NB) rat [44, 45]. PTI-NB rats do not show overt clinical signs of infection but they are significantly smaller than mock-infected littermates. The basis for this abnormal growth is unclear, and PTI-NB rats do not exhibit altered growth hormone function or food consumption. PTI-NB rats exhibit distinct deficiencies in emotional and cognitive functions as well as physiologic and neurodevelopmental abnormalities likely due to selective damage on specific neuronal

populations [45]. BDV infection interferes with the development of CNS areas that experience extensive postnatal maturation. BDV prenatal infection is associated with cerebella hypoplasia and progressive degeneration of the dentate gyrus (DG) in the hippocampus [46–48]. In addition to the abnormalities seen in cerebellum and hippocampus, PTI-NB rats also exhibit significant cortical shrinkage that involves a selective vulnerability of both pyramidal and GABA-ergic cortical neurons [47].

By the completion of development (postnatal day [PND] 21), the cerebellum of PTI-NB rats is smaller than in control rats, with significant thinning of the molecular and internal granule cell layers [45, 49]. Purkinje cells (PkC) are the predominant cell type infected in the cerebellum by PND 8. Cerebellar granule cell neurogenesis and survival depend critically on the integrity of PkC during an early postnatal period [50]. PkC loss in PTI-NB rats is not apparent before PND 27, whereas increased granule cell loss is seen at PND 8. BDV infection of PkC at early times may compromise PkC functions in supporting the multiplication, maturation, and migration of the granular cells.

Astrocytes provide the physical track upon which the granule cells migrate plus neurotropic support to surrounding cells. PTI-NB rats have a very robust astrocytosis. Hence, altered astrocyte physiology in PTI-NB rats could also contribute to disturbances in cerebellar development. BDV-infected neurons in the DG are progressively destroyed [48, 51, 52]. The majority of DG granule neurons are postnatally generated, and neurogenesis occurs in this area well into adulthood. Reduced numbers of DG neurons in PTI-NB rats are first seen about PND 21. Around PND 60, most of the DG has been replaced by glia cells. Massive apoptotic cell death of DG granule cells is observed at postinfection days 27–33 but significantly subsides by PND 48.

BDV-induced changes in CNS gene expression. BDV-induced disturbances of the balance between the expression of pro- and antiapoptotic genes likely contribute to neuronal cell death [48, 53]. Damage to synaptic structures, as determined by reduced expression levels of synaptic proteins GAP-43 and synaptophysin, antecede neuronal loss in the hippocampal and cortical regions of PTI-NB rats [47]. In addition, neurons of PTI-NB rats appear affected in their axonal transport. These findings suggest the possibility that BDV-induced synaptic and axoplasmic flow damage may impair the uptake and trafficking of growth factors required for neuronal function. This together with other factors could contribute to selective neuronal vulnerability and cause localized alterations in neuronal communication resulting in cell injury.

Neurotrophins and their receptors play important roles in survival and differentiation of DG and cerebellar granule cells. Therefore, BDV-induced disturbances in the neurotrophin system may also play a role in DG degeneration, a cerebellar hypoplasia in PTI-NB rats [48, 54]. Cytokines play an important role in CNS function under normal physiologic and under pathologic conditions. Chronic up-regulation of proinflammatory cytokines IL-1α and β, IL-6, and TNF-α [48, 52] and of the chemokines interferon-inducible protein-10 and RANTES [55] is observed in the CNS of PTI-NB rats. Cytokine effects on the brain chemistry and behavioral responses are well documented, suggesting that altered cytokine expression also can contribute to CNS disturbances in PTI-NB rats.

Astrocytes also provide a substrate for neuronal migration during brain development, and the astrocytic network participates in brain information processing in cooperation with neuronal networks. In addition, astrocytes participate in the elimination of neurotoxins, neuronal differentiation, and modulation of neuronal neurotransmitter gene expression. Hence, altered astrocyte physiology may contribute to the complex virus-host interactions leading to CNS abnormalities in PTI-NB rats. Thus, primary cortical astrocytes persistently infected by BDV exhibit a severe and specific impairment in their ability to take up glutamate, which is an important physiologic function for maintaining brain homeostasis [56].

Astrocytes of PTI-NB rat brains also exhibit increased expression levels of tissue factor (TF) [57]. TF is the primary initiator of the coagulation protease cascade that results in the generation of the protease thrombin. Increasing evidence indicates that proteins of the coagulation and fibrinolysis systems may function in the CNS independent of blood clotting, regulating normal brain development and defending the brain against injury-related damage. Altered protease activities may contribute to neuronal damage, and thrombin has been implicated in several neurodegenerative diseases. Of interest, thrombin is predominantly expressed by dopaminergic neurons of the mesencephalon, suggesting a possible link between TF up-regulation and the dopaminergic abnormalities observed in rats with BD.

Behavioral abnormalities. PTI-NB rats exhibit distinct cognitive, behavioral, and physiologic abnormalities [45, 49]. PTI-NB rats show impaired cognitive functions in different learning paradigms, including spatial discrimination learning and spatial learning and memory via navigation based on visual cues. Compared with controls, PTI-NB rats also show deficiencies in contextual fear conditioning and aversive learning, tasks that also provide information about the integrity of the limbic system. Behavioral tests aimed at probing emotional responses and anxiety uncovered chronic emotional abnormalities in PTI-NB rats that are likely related to the hippocampal and cerebellar damage seen in these animals.

Despite their significant cerebellar abnormalities, PTI-NB rats do not show obvious signs of ataxia. However, when subjected to sensitive behavioral tests, they exhibit a variety of locomotor deficits that are likely linked to cerebellar abnormalities [58]. They also display altered sleep-wake cycles and increased preference for salt-containing solutions [44]. Abnormal play behavior also is seen in PTI-NB rats. BDV-infected rats show a significantly decreased drive to engage their partner

in social play [59]. PTI-NB rats have specific neurochemical alterations affecting the presynaptic monoaminergic systems, a finding consistent with the observed behavioral deficits [45, 49].

BDV infection of the neonatal rat as a model of neurodevelopmental disorders. Despite the controversy surrounding the potential association of BDV infection with neuropsychiatric disorders, the spectrum of neurobehavioral and neurodevelopmental alterations seen in PTI-NB rats have remarkable parallels with some of the main the features of human neuropsychiatric disorders. The cerebellar and hippocampal dysgenesis observed in PTI-NB rats resembles neurodevelopmental disturbances documented in schizophrenia [60], autism [61], and affective disorders [62]. The similarities between the signs of autism spectrum disorder and the manifestations of neonatal BDV infection of rats are particularly striking. Thus, the sensorimotor alterations (likely related to cerebellar pathology, abnormal taste preferences, sleep disturbances, and social deficits) observed in autistic young children are recreated to some extent in PTI-NB rats [45, 49].

Outlook

BDV has unique genetic and biologic features that represent the prototypic member of a new group of RNA animal viruses. Therefore, the investigation of its molecular and cellular biology may provide new insights about virus-host interactions underlying persistence and associated disease. As with other mononegaviruses, these investigations of the molecular biology of BDV will greatly benefit from the establishment of a reverse genetic system. Evidence suggests that BDV might infect humans. However, the prevalence of BDV in humans and its possible association with certain neuropsychiatric disorders remain to be solved. Increased knowledge about the molecular and cellular biology of BDV should help in the development of standardized sensitive and reliable serologic and nucleic acid diagnostic tests to address this pressing question.

The PTI-NB rat model can facilitate experimental work aimed at understanding the bases for the neurostructural, locomotor, cognitive, and social deficits associated with certain human mental disorders. Novel techniques for the analysis of global gene expression in the PTI-NB rat model may identify molecular pathways underlying these deficits and could assist the design of new therapies for these very serious diseases.

References

Note. Space limitations precluded the citation of all pertinent references. Instead only recent reviews are cited.

1. Mohammed AM, Norrby E, Kristensson K. Viruses and behavioral changes: a review of clinical and experimental findings. Rev Neurosci **1993**;4: 267–80.
2. Yolken RH, Fuller Torrey E. Viruses, schizophrenia, and bipolar disorder. Clin Microbiol Rev **1995**;8:131–45.
3. Ahmed R, Morrison LA, Knipe DM. Viral persistence. In: Nathanson N, ed. Viral pathogenesis. Philadelphia: Lippincott-Raven, **1997**:181–98.
4. de la Torre JC, Borrow P, Oldstone MB. Viral persistence and disease: cytopathology in the absence of cytolysis. Br Med Bull **1991**;47:838–51.
5. ter Meulen V. Virus-cell interactions in the nervous system. Semin Neurosci **1991**;3:81–173.
6. de la Torre JC. Molecular biology of Borna disease virus: prototype of a new group of animal viruses [review]. J Virol **1994**;68:7669–75.
7. Schneemann A, Schneider PA, Lamb RA, Lipkin WI. The remarkable coding strategy of Borna disease virus: a new member of the nonsegmented negative strand RNA viruses [review]. Virology **1995**;210:1–8.
8. Rott R, Becht H. Natural and experimental Borna disease in animals. Curr Top Microbiol Immunol **1995**;190:17–30.
9. Carbone KM. Borna disease virus and human disease. Clin Microbiol Rev **2001**;14:513–27.
10. Lipkin WI, Schneemann A, Solbrig MV. Borna disease virus: implications for human neuropsychiatric illness [review]. Trends Microbiol **1995**;3: 64–9.
11. de la Torre JC, Carbone KM, Lipkin WI. Molecular characterization of the Borna disease agent. Virology **1990**;179:853–6.
12. Gonzalez-Dunia D, Sauder C, de la Torre JC. Borna disease virus and the brain. Brain Res Bull **1997**;44:647–64.
13. Kohno T, Goto T, Takasaki T, et al. Fine structure and morphogenesis of Borna disease virus. J Virol **1999**;73:760–6.
14. Zimmermann W, Breter H, Rudolph M, Ludwig H. Borna disease virus: immunoelectron microscopic characterization of cell-free virus and further information about the genome. J Virol **1994**;68:6755–8.
15. Staeheli P, Sauder C, Hausmann J, Ehrensperger F, Schwemmle M. Epidemiology of Borna disease virus. J Gen Virol **2000**;81:2123–35.
16. Sierra-Honigmann AM, Rubin SA, Estafanous MG, Yolken RH, Carbone KM. Borna disease virus in peripheral blood mononuclear and bone marrow cells of neonatally and chronically infected rats. J Neuroimmunol **1993**;45:31–2.
17. Stitz L, Dietzschold B, Carbone KM. Immunopathogenesis of Borna disease. Curr Top Microbiol Immunol **1995**;190:75–92.
18. Carbone KM, Moench TR, Lipkin WI. 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 **1991**;50:205–14.
19. Gonzalez-Dunia D, Cubitt B, de la Torre JC. Mechanism of Borna disease virus entry into cells. J Virol **1998**;72:783–8.
20. Gonzalez-Dunia D, Cubitt B, Grasser FA, de la Torre JC. Characterization of Borna disease virus p56 protein, a surface glycoprotein involved in virus entry. J Virol **1997**;71:3208–18.
21. Perez M, Watanabe M, Whitt MA, de la Torre JC. N-terminal domain of Borna disease virus G (p56) protein is sufficient for virus receptor recognition and cell entry. J Virol **2001**;75:7078–85.
22. Schneemann A, Schneider PA, Kim S, Lipkin WI. Identification of signal sequences that control transcription of Borna disease virus, a nonsegmented, negative-strand RNA virus. J Virol **1994**;68:6514–22.
23. Cubitt B, Ly C, de La Torre JC. Identification and characterization of a new intron in Borna disease virus. J Gen Virol **2001**;82:641–6.
24. Tomonaga K, Kobayashi T, Lee BJ, Watanabe M, Kamitani W, Ikuta K. Identification of alternative splicing and negative splicing activity of a nonsegmented negative-strand RNA virus, Borna disease virus. Proc Natl Acad Sci USA **2000**;97:12788–93.
25. Loh LC, Locke D, Melnychuk R, Lafertet S. The RGD sequence in the cytomegalovirus DNA polymerase accessory protein can mediate cell adhesion. Virology **2000**;272:302–14.
26. Jehle C, Lipkin WI, Staeheli P, Marion RM, Schwemmle M. Authentic Borna disease virus transcripts are spliced less efficiently than cDNA-derived viral RNAs. J Gen Virol **2000**;81:1947–54.
27. Kobayashi T, Watanabe M, Kamitani W, Tomonaga K, Ikuta K. Translation initiation of a bicistronic mRNA of Borna disease virus: a 16-kDa phos-

phoprotein is initiated at an internal start codon. Virology **2000**;277:296–305.
28. Schneider PA, Kim R, Lipkin WI. Evidence for translation of the Borna disease virus G protein by leaky ribosomal scanning and ribosomal reinitiation. J Virol **1997**;71:5614–9.
29. Whittaker GR, Helenius A. Nuclear import and export of viruses and virus genomes. Virology **1998**;246:1–23.
30. Kobayashi T, Shoya Y, Koda T. Nuclear targeting activity associated with the amino terminal region of the Borna disease virus nucleoprotein. Virology **1998**;243:188–97.
31. Shoya Y, Kobayashi T, Koda T, Ikuta K, Kakinuma M, Kishi M. Two proline-rich nuclear localization signals in the amino- and carboxyl-terminal regions of the Borna disease virus phosphoprotein. J Virol **1998**;72:9755–62.
32. Wolff T, Unterstab G, Heins G, Richt JA, Kann M. Characterization of an unusual importin alpha binding motif in the Borna disease virus p10 protein that directs nuclear import. J Biol Chem **2002**;277:12151–7.
33. Wolff T, Pfleger R, Wehner T, Reinhardt J, Richt JA. A short leucine-rich sequence in the Borna disease virus p10 protein mediates association with the viral phospho- and nucleoproteins. J Gen Virol **2000**;81:939–47.
34. Compans RW, Melsen LR, de la Torre JC. Virus-like particles in MDCK cells persistently infected with Borna disease virus. Virus Res **1994**;33:261–8.
35. Carbone KM, Duchala CS, Griffin JW, Kincaid AL, Narayan O. 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 **1987**;61:3431–40.
36. Gosztonyi G, Ludwig H. Borna disease—neuropathology and pathogenesis. Curr Top Microbiol Immunol **1995**;190:39–73.
37. Stitz L, Noske K, Planz O, Furrer E, Lipkin WI, Bilzer T. A functional role for neutralizing antibodies in Borna disease: influence on virus tropism outside the central nervous system. J Virol **1998**;72:8884–92.
38. Bilzer T, Stitz L. Immunopathogenesis of virus diseases affecting the central nervous system. Crit Rev Immunol **1996**;16:145–222.
39. Neumann H, Schmidt H, Wilharm E, Behrens L, Wekerle H. Interferon gamma gene expression in sensory neurons: evidence for autocrine gene regulation. J Exp Med **1997**;186:2023–31.
40. Solbrig MV, Koob GF, Joyce JN, Lipkin WI. A neural substrate of hyperactivity in Borna disease: changes in brain dopamine receptors. Virology **1996**;222:332–8.
41. Lipkin WI, Hatalski CG, Briese T. Neurobiology of Borna disease virus. J Neurovirol **1997**;3:S17–20.
42. Gies U, Bilzer T, Stitz L, Staiger JF. Disturbance of the cortical cholinergic innervation in Borna disease prior to encephalitis. Brain Pathol **1998**;8:39–48.
43. Hatalski CG, Hickey WF, Lipkin WI. Evolution of the immune response in the central nervous system following infection with Borna disease virus. J Neuroimmunol **1998**;90:137–42.
44. Bautista JR, Schwartz GJ, de la Torre JC, Moran TH, Carbone KM. Early and persistent abnormalities in rats with neonatally acquired Borna disease virus infection. Brain Res Bull **1994**;34:31–40.
45. Pletnikov MV, Moran TH, Carbone KM. Borna disease virus infection of the neonatal rat: developmental brain injury model of autism spectrum disorders. Front Biosci **2002**;7:D593–607.
46. Bautista JR, Rubin SA, Moran TH, Schwartz GJ, Carbone KM. Developmental injury to the cerebellum following perinatal Borna disease virus infection. Brain Res Dev Brain Res **1995**;90:45–53.
47. Gonzalez-Dunia D, Watanabe M, Syan S, Mallory M, Masliah E, de la Torre JC. Synaptic pathology in Borna disease virus persistent infection. J Virol **2000**;74:3441–8.
48. Hornig M, Weissenbock H, Horscroft N, Lipkin WI. An infection-based model of neurodevelopmental damage. Proc Natl Acad Sci USA **1999**;96:12102–7.
49. Hornig M, Solbrig M, Horscroft N, Weissenbock H, Lipkin WI. Borna disease virus infection of adult and neonatal rats: models for neuropsychiatric disease. Curr Top Microbiol Immunol **2001**;253:157–77.
50. Smeyne RJ, Chu T, Lewin A, et al. Local control of granule cell generation by cerebellar Purkinje cells. Mol Cell Neurosci **1995**;6:230–51.
51. Rubin SA, Sylves P, Vogel M, et al. Borna disease virus-induced hippocampal dentate gyrus damage is associated with spatial learning and memory deficits. Brain Res Bull **1999**;48:23–30.
52. Sauder C, de la Torre JC. Cytokine expression in the rat central nervous system following perinatal Borna disease virus infection. J Neuroimmunol **1999**;96:29–45.
53. Weissenbock H, Hornig M, Hickey WF, Lipkin WI. Microglial activation and neuronal apoptosis in Bornavirus infected neonatal Lewis rats. Brain Pathol **2000**;10:260–72.
54. Zocher M, Czub S, Schulte-Monting J, de La Torre JC, Sauder C. Alterations in neurotrophin and neurotrophin receptor gene expression patterns in the rat central nervous system following perinatal Borna disease virus infection. J Neurovirol **2000**;6:462–77.
55. Sauder C, Hallensleben W, Pagenstecher A, et al. Chemokine gene expression in astrocytes of Borna disease virus-infected rats and mice in the absence of inflammation. J Virol **2000**;74:9267–80.
56. Billaud JN, Ly C, Phillips TR, de la Torre JC. Borna disease virus persistence causes inhibition of glutamate uptake by feline primary cortical astrocytes. J Virol **2000**;74:10438–46.
57. Gonzalez-Dunia D, Eddleston M, Mackman N, Carbone K, de la Torre JC. Expression of tissue factor is increased in astrocytes within the central nervous system during persistent infection with Borna disease virus. J Virol **1996**;70:5812–20.
58. Pletnikov MV, Rubin SA, Carbone KM, Moran TH, Schwartz GJ. Neonatal Borna disease virus infection (BDV)-induced damage to the cerebellum is associated with sensorimotor deficits in developing Lewis rats. Brain Res Dev Brain Res **2001**;126:1–12.
59. Pletnikov MV, Rubin SA, Vasudevan K, Moran TH, Carbone KM. Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: a model of autism. Behav Brain Res **1999**;100:43–50.
60. Altshuler LL, Conrad A, Kovelman JA, Scheibel A. Hippocampal pyramidal cell orientation in schizophrenia. A controlled neurohistologic study of the Yakovlev collection. Arch Gen Psychiatry **1987**;44:1094–8.
61. Kemper TL, Bauman ML. The contribution of neuropathologic studies to the understanding of autism. Neurol Clin **1993**;11:175–87.
62. Soares JC, Mann JJ. The anatomy of mood disorders—review of structural neuroimaging studies. Biol Psychiatry **1997**;41:86–106.