Borna disease: virus-induced neurobehavioral disease pathogenesis

Kathryn M Carbone*†‡, Steven A Rubin*, Yoshii Nishino* and Mikhail V Pletnikov*‡

Studies of the pathogenesis of neurobehavioral diseases following Borna disease virus infections have been increasing rapidly over the past ten years. Recent major advances have included a report of vertical transmission of the virus in its natural host, the horse, and a report of isolation of a novel variant, No/98, in that same species. In rats infected neonatally with the Borna disease virus that lack blood-borne inflammation in the brain, evidence of an 'endogenous' brain inflammatory response is abundant, with elevated expression of cytokine and chemokine mRNA. Infection in these rats is also associated with abnormal levels of neurotransmitters, including serotonin and norepinephrine. Data and debate continue to be forthcoming about the role of Borna disease virus in human infection and psychiatric disease.

Addresses
*FDA/CBER, Laboratory of Pediatric and Respiratory Viral Diseases, HFM 460, 8800 Rockville Pike, Bethesda, Maryland 20892, USA, and Departments of Medicine† and Psychiatry‡, The Johns Hopkins University School of Medicine, 618 Ross, 720 Rutland Avenue, Baltimore, Maryland 21205, USA.
†e-mail: Carbonek@cber.fda.gov

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Abbreviations

BD	Borna disease
BDV	Borna disease virus
MHC	major histocompatibility complex
NGF	nerve growth factor
PND	post-natal day
RT-PCR	reverse transcription PCR

Introduction

Borna disease virus (BDV) is the prototype and sole member of the Bornaviridae family in the Mononegavirales order of viruses. This small, negative-sense single-strand RNA virus has an 8.9 kb genome that encodes for some unusual biological features, such as a wide host range (birds to mammals), nonlytic replication and persistent infection, replication in the cell nucleus, strong neurotropic properties (replication in neurons, astrocytes, Schwann cells and oliogodendroglia), and production of potent immunopathogenetic-based neurobehavioral disease [1]. It is not confirmed whether or not BDV infects humans; this and whether or not it causes psychiatric disorders in humans have been the causes of highly controversial debate over the past 15 years. In this review, we shall discuss recent advances in natural and experimental host–virus interactions, emphasising disease pathogenesis.

Transmission

Although Borna disease (BD) has been diagnosed in horses and sheep in Central Europe for over 100 years, there is still much to learn about natural BDV infection. For example, although it is assumed that intranasal spread of BDV is a likely mode of virus infection, the specific mechanism for horizontal transmission has not been demonstrated. Moreover, the possibility of vertical transmission remains controversial. New reverse transcription PCR (RT-PCR) and *in situ* hybridization evidence of BDV in a fetus from a horse with wild-type BDV infection suggests intrauterine transmission of BDV in nature [2].

BDV genome variability and effects on diagnosis

For an RNA virus, BDV has an extremely uniform genetic sequence among the various isolates published in the literature (typically less than 5% heterogeneity). It is tempting to speculate that some of the invariability in the BDV genome may relate to the preference of this virus to replicate in the same tissue (e.g. the nervous system) in a variety of different hosts. In addition, studies suggest that there is resistance to superinfection by cells persistently infected by BDV, thus limiting the opportunities for growth and spread of variant strains within the infected organism [3••].

BDV scientists have taken advantage of the uniformity of the BDV genome to apply 'universal' reagents (e.g., oligonucleotide primers for RT-PCR, antibodies and recombinant BDV antigens) for BDV detection to a variety of research settings, from testing infected cells in culture to screening human samples. However, in certain species (e.g. cat and human), recovery of BDV using these standard approaches has been difficult. Perhaps some of this difficulty is related to the existence of unexpected 'nonstandard' strains of BDV that are not detected by standard reagents. These new strains may contain genome changes that facilitate replication in specific hosts. For example, after inoculation of a mixture of two BDV strains into mice, preferential replication of one of the strains was demonstrated [3••].

Additional information suggests that these 'crossreactive' reagents may not identify every case of BDV infection. A new BDV strain, No/98, was isolated from a pony in Austria with substantial genome sequence deviation (>15%) from the known reference strains, V and He80 [4•]. As a result, No/98 was difficult to detect using standard diagnostic RT-PCR protocols. This finding brings into question assumptions of sensitivity and specificity of current reagents for novel isolates and the meaning of negative findings in the search for evidence of human BDV infection.

Figure 1

Cerebellum from Lewis rats intracranially inoculated with **(a)** uninfectious control material on PND 1; **(b)** BDV on PND 1; and **(c)** BDV on PND 15 (cyclosporin A treated to suppress encephalitis). Note cerebellar dysplasia in (b), and normal appearance of cerebellum in (c), illustrating the influence of critical periods of brain development on virus-induced neurodevelopmental damage. Paraffin embedded saggittal section of cerebellum, 10m-thick, stained with hematoxylin and eosin. Photographed by SA Rubin.

Immunopathogenic Borna disease

The extraordinary importance of the host's role in defining the clinical expression of BD has been a consistent finding in BDV research. BD was originally described as an acute and fatal encephalitis of farm animals, causing hyperactivity, paralysis and ataxia [5]. It was recognized early that the incapacitating neurological disease of classical BD was largely the result of the BDV-induced encephalitis, that is, nervous system damage due to blood-borne inflammatory cell injury to the infected brain [1]. With such a fascinating disease model, it is not surprising that, until recently, the majority of BDV research was centered on infection and BD in immunocompetent hosts.

It has been well established that $CD8^+$ T-lymphocytes play a significant role in destroying BDV-infected neurons and producing the immunopathogenic BD [1]. Planz *et al.* [6••] used the BDV-infected rat model to identify the first naturally processed peptide synthesized by a virus — ASYAQMTTY from BDV nucleoprotein (p40) — that is recognized by classical $CD8^+$ T-cells in association with the $RT1.A^1$ (a type of rat major histocompatibility complex [MHC] molecule that is part of the classical class Ia and is responsible for conventional antigen presentation to cytotoxic T lymphocytes).

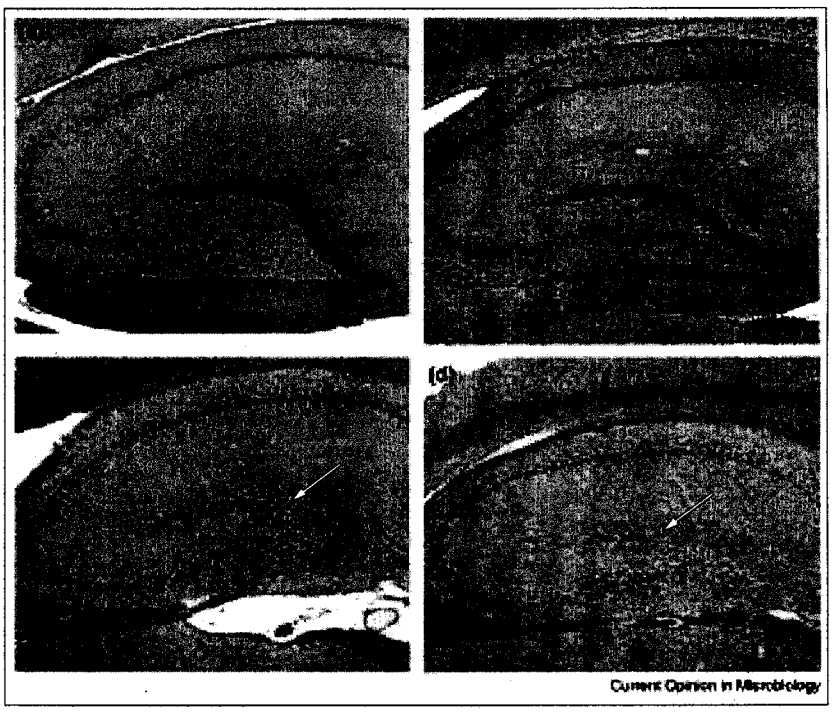
Although the cellular immune response is known to play the major role in classical BD, the role of antibodies in protection from infection and/or promotion of disease is not as well understood. Furrer *et al.* [7] describe *in vivo* neutralizing activity by monoclonal antibodies specific for the major glycoprotein (gp94), thereby suggesting the potential for an effective vaccine against BDV.

Other forms of Borna disease

We now know that BDV produces a myriad of disease syndromes, including asymptomatic infection, phasic or chronic behavioral disease and phasic severe neurological disease, in addition to the rapidly fatal neurological signs of classical BD [1]. BD expression varies not only by species but also by strain within species. Within a single species or strain of animal, BD may fluctuate with the age of the host at the time of infection. The specific reasons for these variations in BD expression are not known.

A great deal has been learnt about BD pathogenesis by studies using BDV infection in experimental animal models, such as the rat model. A new animal model of BDV infection — infection of newborn gerbils — has now been developed with distinct clinical and pathological features [8,9]. Like newborn rats, newborn gerbils are highly susceptible to BDV infection and develop minimal inflammatory responses in the brain, but, in contrast to the apparently immunotolerant behavioral disease following infection of newborn rats, gerbils develop severe neurological disease. Because there is extensive evidence of 'endogenous' immune reaction to BDV in rats, the gerbil model will provide another valuable setting with which to

Figure 2



Hippocampus from Lewis rats intracranially inoculated with **(a)** uninfected control material on PND 1 at age 60 days; **(b)** BDV on PND 1 at age 21 days; **(c)** BDV on PND 1 at age 75 days; and **(d)** BDV on PND 15 at age 70 days (cyclosporin A treated to suppress encephalitis). Note dentate gyrus present in (a) and (b) at early timepoint of infection (black arrows), and dissolution of dentate gyrus in late infection in (c) and (d) (white arrows). This shows that gradual loss of dentate gyrus neurons occurs regardless of whether the rat is neonatal or infant (PND 15) at the time of infection. Paraffin-embedded saggital section of hippocampus, 10m-thick, stained with hematoxylin and eosin. Photographed by SA Rubin.

investigate the link between viral infection of the central nervous system and neurological damage, in the setting of limited blood-borne inflammatory infiltrates.

Behavioral diseases caused by Borna disease virus infection

There were a few early hints that BDV infection could lead to inapparent infection and subtle behavioral disease [10]. In contrast to classical immunopathogenic BD with encephalitis seen in horses, sheep and adult infected rats, other forms of BD seen in tree shrews and rats infected neonatally included understated signs of behavioral disease that were typically (but not always [11]) expressed without encephalitis.

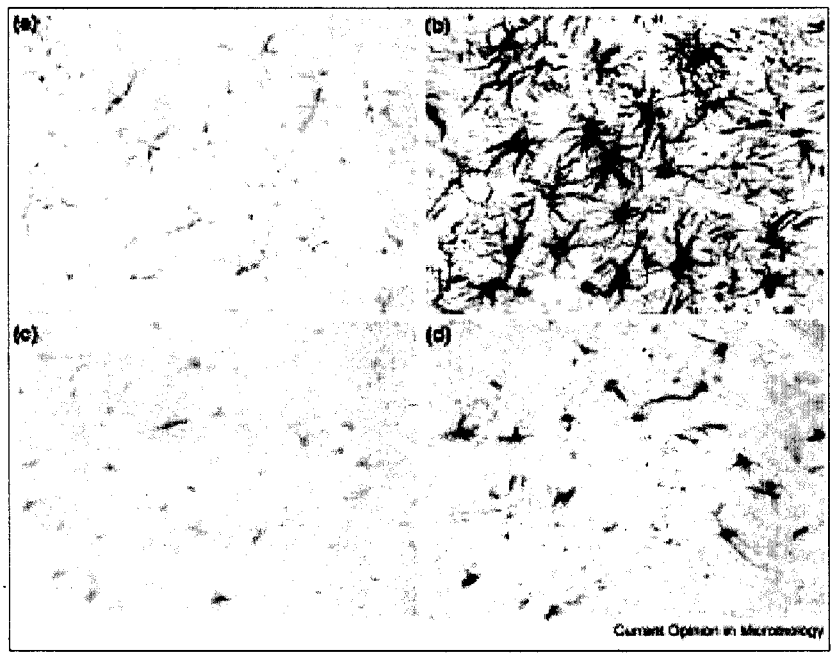
Perhaps due to the specialized nature of behavioral science, experimental behavioral testing was initially not often found in virology research laboratory settings. Models with inconspicuous behavioral forms of BD required formal behavioral evaluations and, thus, were less appealing to the majority of BDV virologists. Whatever the reason, for many years, there were few research articles published about the more behavioral models of BD.

Led by the important early contributions of a few laboratories studying noninflammatory BDV infection (e.g., BDV infection of the neonatal rat [12–15]), BD pathogenesis models now, fortunately, are recognised to provide a tremendous opportunity to study the direct effects of virus infection of the brain in the absence of a blood-borne cellular immune response. In addition, studies in the rat infected neonatally with BDV have been extremely useful in modeling neurodevelopmental diseases of infectious and noninfectious etiologies.

Developmental effects of persistent BDV infection on the nervous system

Perhaps one of the most novel aspects of BD pathogenesis studies focuses on the effects of persistent BDV infection on nervous system development and function. Viruses are known to be potent nervous system teratogens (which cause changes in normal development during fetal growth, leading to birth defects or congenital defects) and disease outcomes depend on host contributions in addition to the specific infectious agent. Advances have been made since the first identification of cerebellar, hippocampal [15] (Figures 1 and 2) and cortical pathology [16] in rats infected neonatally with BDV. Differences in neuroanatomical and behavioral outcomes after early BDV infection can be linked to the relative maturity of neural circuits at the time of infection [17]. When the rats are infected during cerebellar development (post-natal day [PND]1), cerebellar dysplasia (abnormal cerebellar development) occurs (Figure 1) and hyperactivity ensues. When they are infected after cerebellar development has largely concluded (PND 15), no cerebellar defects are seen (Figure 1) and the rats have normal activity levels. In contrast to the cerebellum, hippocampus development continues throughout life in the rat and, thus, infections both at PND 1 and PND 15 lead to gradual dissolution of the hippocampal dentate

Figure 3



Matched sections of brains from Lewis rats intracranially inoculated with **(a,c)** uninfected control material on PND 1; and **(b,d)** BDV on PND 1. Sections are immunohistochemically stained with (a,b) antibody to GFAP, showing reactive astrocytes in BDV-infected rat, or (c,d) OX-42 (a microglial marker), showing activated microglia in BDV-infected rat. Paraffin-embedded saggital sections, 10m-thick. Photographed by SA Rubin.

gyrus neurons (Figure 2). These findings demonstrate a link between variability in neuroanatomical damage or disease expression and infection at different critical periods of brain development.

It has been difficult to separate the roles of inflammatory cells, virus, neurotransmitter toxicity, neurotrophins and soluble immune mediators in BDV-associated neurological damage. In the absence of blood-borne inflammation in the neonatally infected rat, there is an opportunity to evaluate the neuropathological outcomes. In addition to the previously described changes in the cerebellum, hippocampus and cortex, apoptotic cells were seen in the hippocampal dentate gyrus and cortical layers five and six, using terminal deoxynucleotidylatransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay (see [18] for details).

An 'endogenous' neural cell immune response

The ability to induce a BDV brain infection without encephalitis permits the study of an 'endogenous' neural cell immune response. There is only limited and transient evidence of inflammatory cells in the brain of the rat infected nenonatally with BDV [19,20••]. However, infected rats have intense microglial cell activation (Figure 3) and transient expression of MHC class I and class II [18]. In neonatal BDV infection, the presence of reactive astrocytes (astrogliosis) has been known for years [21] (Figure 3), but recent publications expand our knowledge about the brain's response to noninflammatory BDV infection. (Reactive astrocytes signify astrocytosis, a response to infection or injury to the nervous system in which astrocytes enlarge and express greater amounts of glial fibrillary acidic protein [GFAP], an astrocyte marker) Some recent findings are summarized in Table I, and include the presence of regional and developmental abnormalities in mRNA expression of cytokines, chemokines, neurotransmitters, apoptosis genes, neurotrophins and other neuroactive substances [18,20••,22–25].

In rats infected neonatally with BDV, there is upregulation of proinflammatory cytokines (e.g. IL-6, TNF-α, IL-1α and IL-1β), suppressive cytokines (e.g. TGF-β1) and chemokines (IP-10 and RANTES). Abnormalities in cytokine expression are a dynamic modality, varying by region examined and time post-inoculation. Cytokines such as IL-1β and TNF-α are potent inducers of astrocytosis and may induce some of the observed behavioral and functional deficits, such as abnormal learning and motor activity, decreased gain in body weight and changes in taste preferences [25]. Conversely, TGF-β1 inhibits cytokine release and astrocyte proliferation, suggesting a negative feedback regulatory response, although these responses are transient [20••] and are apparently inadequate to control the stimulatory cytokine responses to BDV. Chemokine mRNAs were also detected in animals that were genetically altered to lack MHC class I expression and B and/or T-lymphocytes, suggesting a direct stimulation of chemokine synthesis by BDV infection [23]. Astrocytes, including the Bergmann glia of the cerebellum, were found to be a major source of IP-10 in BDV-infected rats [23]. In the first clear association of an immune mediator with functional damage in BDV-infected mice, elevated

mRNA expression of IP-10 and RANTES was associated with behavioral abnormalities, including abnormalities in spatial learning and memory [26••].

Neurotrophin upregulation is a common response to injury in the nervous system. Since early reports of interactions between BDV and nerve growth factor (NGF) *in vitro* [27], several authors have begun to investigate neurotrophin interaction with BDV infection. Declines in mRNA transcripts for brain-derived neutrophic factor (BDNF), NGF and neurotrophin-3 (NT3) over time were reported in the hippocampi of rats infected neonatally with BDV [20••,24]. Passage of BDV-infected PC-12 cells selected a phenotype of cell that was less responsive to NGF, downregulated the expression of molecules involved in neural plasticity (e.g. synatophysin and growth-associated protein-43), and caused constitutive activation of the ERK1/2 pathway with reduced efficiency of translocation to the nucleus [28].

Neurotransmitter toxicity may be another contributor to the damage observed in the neonatally infected rats. BDV antigen is found in regions rich in excitatory neurotransmitters [29], and is expressed in astrocytes responsible for maintaining brain homeostasis [21,30]. It has now been demonstrated *in vitro* that BDV infection of cortical astrocytes leads to severe impairment in their ability to take up and detoxify glutamate, with potential consequences of excitatory neurotoxicity [31•]. This finding suggests a potentially significant mechanism for BDV-associated toxicity related to astrocyte infection and dysfunction.

BD models of human disease

In the 1960s, virus-induced autistic disease was reported following intrauterine rubella infection [32], and the model of the rat infected neonatally with BDV was first developed in 1983 [12,13]. Initially, following the identification of behavioral disease in these animals [14], only a few laboratories continued work with this model. Over 15 years later, as outlined in [33], the evidence accumulated about this model has demonstrated a startling consistency between neonatal BDV infection in rats and autism spectrum disease in children. The three hallmark features of autism were found in rats infected neonatally with BDV and established the validity of the BDV/rat-autism model. These features are: developmental damage to the cerebellum (dysplasia) and hippocampus (dentate gyrus dissolution) [15,34,35]; abnormal social behavior as evidenced by deficits in play behavior [36••]; and serotonin neurotransmitter abnormalities [37••].

Recently, several laboratories have provided additional important information about this model system, including information on neuroanatomical, neuroimmune, neurotrophin and behavioral abnormalities. New information on neuroanatomical abnormalities includes gradual loss of Purkinje cells in the cerebellum beginning around one month after infection [24,38]. Novel behavioral deficits recently reported include chronic emotional abnormalities [39] and abnormal sensorimotor behavior development [20••,40].

Humans and Borna disease virus infection

A search for the 'human BDV equivalent' has been underway for over 15 years [41]. Controversy abounds regarding the testing of humans for evidence of past or current BDV infection, including patient and control selection and evaluation, testing methods (e.g. serology versus virus sequence isolation) and interpretation of results. Perhaps the difficulty in detecting infectious BDV is due to sampling error, according to a new report of the repeated isolation of BDV from the granulocyte fraction of peripheral white blood cells (PBMC) [42]. Before this report, investigators had looked for evidence of BDV or BDV sequences in the mononuclear cell fraction of PBMC. It is also possible that scientists are not using the best testing paradigms or techniques to identify human BDV. For the first time, scientists have utilized a large set of tests (e.g. three separate serological assays: immunofluorescence (IF), western blot (WB) and ECLIA [43]) combined with cellular immune responses to BDV, to look for evidence of BDV infection in a variety of psychiatric patients, such as those with mood disorder and schizophrenia [44••]. As controls, age- and sex-matched blood donors were used. Although the percentages of positive results in patients and controls were not statistically significant, it is noteworthy that the scientists identified one mood disorder patient with T-lymphocyte responses to two major BDV antigens (p40 and p24) who was also WB-, ECLIA- and IF-positive for anti-BDV antibody. Considering no viral sequences were recovered by RT-PCR, this patient could have been infected with BDV and could have recovered with virus clearance (although the clinical pattern of human BDV infection is not known).

Although infectious BDV has been recovered from patients with schizophrenia [45], it is not surprising that studies have failed to show a causal association between a specific human disease and evidence of BDV infection. First, scientists lack a validated test with which to identify BDV infection in the human. Second, proper patient selection and assessment are critical for drawing causative links between infections and disease. At this time, it is unclear which disease (e.g. affective disorder or schizophrenia) or which subset of patients with a specific disease are likely candidates for a BDV infectious etiology.

Homogeneity among BDV strains has complicated the search for human BDV infection. For example, the actual source (e.g. human isolate or contaminating laboratory strain) of BDV RT-PCR sequences and infectious isolates obtained from human tissues is being hotly debated [46–49]. Schwemmle *et al.* have published an intriguing hypothesis that the sequence similarities in selected regions of virus genome among laboratory BDV strains and human strains isolated in those laboratories suggests an inadvertent contamination of human samples with laboratory strains. In

Table 1

Studies of important mediators in brains of rats infected neonatally with BDV.

Compound	Change	Location	Details	Assay	Reference
Immune mediators					
IL-1α mRNA	↑	Hippocampus	2–3X increase from PND 8 to 4.5 months	RPA	[19]
		Cortex	2–3X increase, peak at PND 22–33, decline to normal levels by PND 75		
		Cerebellum	2–3X increase, peak at PND 22–33, decline to normal levels by PND 75		
IL-1α mRNA	↑	Cerebellum	At 4 weeks pi	RPA	[20••]
IL-1β mRNA	↑	Hippocampus	2-6X increase from PND 8 to 4.5 months	RPA	[19]
		Cortex	2-6X increase, peak at PND 22–33, decline by PND 75, but still higher than normal		
		Cerebellum	4-10X increase, remained high at 4.5 months pi		
IL-1β mRNA	↑	Cerebellum, cortex, hippocampus, hypothalamus	At PND 7 and 28	RPA	[25]
IL-1β mRNA	↑	Cerebellum	At 4 weeks pi	RPA	[20••]
IL-1Ra mRNA	↑	Cerebellum	Higher on PND 7 than on PND 28, no significant difference in cortex, hippocampus, hypothalamus	RPA	[25]
IL-1RI mRNA	↑	Cerebellum		RPA	[25]
IL-1R AcP II mRNA	↑	Cerebellum		RPA	[25]
IL-1R AcP I mRNA	–	Cerebellum, cortex, hippocampus, hypothalamus	On PND 7 and 28	RPA	[25]
IL-2 mRNA	–	Brain		RPA	[20••]
IL-3 mRNA	–	Brain		RPA	[20••]
IL-4 mRNA	–	Brain		RPA	[20••]
IL-5 mRNA	–	Brain		RPA	[20••]
IL-6 mRNA	↑	Hippocampus	Increased from PND 8 to 4.5 months	RPA	[19]
		Cortex	Detected PND 8, peaked at PND 22–33, declined to normal levels by PND 75		
		Cerebellum	Slightly elevated compared to controls		
IL-6 mRNA	↑	Cerebellum	At 4 weeks pi	RPA	[20••]
IL-10 mRNA	–	Brain		RPA	[20••]
TNF-α mRNA	↑	Hippocampus	2–6X increase from PND 8 to 4.5 months	RPA	[19]
		Cortex	2–3X increase, peak at PND 22–33, declined by PND 75, but still higher than normal at PND 75		
		Cerebellum	6–8X increase, remained high at 4.5 months pi		
TNF-α mRNA	↑	Cerebellum	At 4 weeks pi		[20••]
TNF-α mRNA	↑	Cerebellum and hypothalamus	At PND 7		[25]
		Cerebellum, cortex, hippocampus and hypothalamus	At PND 28; highest in cerebellum		
TNF-β mRNA	↑	Cerebellum	2.5X increase at 6 weeks pi		[20••]
TNF-β mRNA	↓	Cerebellum	9.5X decrease at 12 weeks pi		[20••]
		Cortex	3.5X decrease at 6 weeks pi		
		Amygdala	12.5X decrease at 2 and 4X decrease at 4 weeks pi		
IFN-γ mRNA	–	Brain			[20••]
TGF-β1 RNA	↑	Cerebellum, cortex, hippocampus, hypothalamus	At PND 7 and 28		[25]
Chemokines					
IP-10 mRNA	↑	Cortex	Very high at PND 22, rapid decline by PND 33, but still significantly higher than uninfected	RPA	[23]
		Cerebellum	Peak at PND 33, gradual decline by PND 75, but still significantly higher than uninfected		
		Hippocampus			
RANTES mRNA	↑	Cortex	Slightly increased at PND 22 and gradual decline to normal by PND 135	RPA	[23]

Table 1 (continued)

		Cerebellum	Gradual increase to peak at PND 48, gradual decline later, but still significantly higher than uninfected by PND 135		
		Hippocampus			
MCP-1 mRNA	-	Cortex, cerebellum		RPA	[23]
MIP-1β mRNA	-	Cortex, cerebellum		RPA	[23]
Lymphotactin mRNA	-	Cortex, cerebellum		RPA	[23]
Neuroactive molecules					
Neuropeptide Y mRNA	↑	Cortex, hippocampus, hypothalamus	At PND 28	RPA	[25]
Pro-opiomelanocortin mRNA (opiod peptide precursor)	↓	Hypothalamus	PND 28	RPA	[25]
Dynorphin mRNA	-	Cerebellum, cortex, hippocampus, hypothalamus		RPA	[25]
Leptin receptor mRNA	-	Cerebellum, cortex, hippocampus, hypothalamus		RPA	[25]
5-HT Concentration	↓	Hippocampus	Decreased at PND 8	HPLC	[36••]
5-HT Concentration	↑	Cortex Cerebellum Hippocampus	Increased at PND 21, 60 and 90 Increased at PND 60 and 90 Increased at PND 21, 60 and 20	HPLC	[36••]
5-HT Concentration	-	Striatum		HPLC	[36••]
NE Concentration	↑	Cortex, cerebellum	At PND 60 and 90	HPLC	[36••]
NE Concentration	-	Hippocampus		HPLC	[36••]
NE Concentration	-	Hypothalamus		HPLC	[36••]
Dopamine concentration	-	Cortex, striatum, hypothalamus		HPLC	[36••]
Neurotrophins					
NGF mRNA	↓	Hippocampus	Decreased 2X between PND 14 to 48	RPA	[24]
BDNF mRNA	↓	Hippocampus	Decreased 2X between PND 21 and 48; no significant difference in cerebellum	RPA	[24]
BDNF mRNA	-	Cerebellum		RPA	[24]
TrkB mRNA (BDNF receptor)	↓	Cerebellum		RPA	[24]
NT-3 mRNA	↓	Hippocampus	Gradual decline to 3X decrease by PND 48	RPA	[24]
NT-3 mRNA	-	Cerebellum		RPA	[24]
TrkC mRNA (NT-3 receptor)	↓	Hippocampus dentate gyrus Cerebellum		RPA	[24]
IGF-1 mRNA	-	Hippocampus		RPA	[24]
BFGF mRNA	-	Hippocampus		RPA	[24]
Apoptosis-related genes					
FAS mRNA	↑	Brain	At 4 weeks pi	RPA	[20••]
Caspase-1 mRNA	↑	Brain	At 4 weeks pi	RPA	[20••]
Caspase-3 mRNA	-	Brain		RPA	[20••]
Bcl-x (L) mRNA	↓	Hippocampus Cerebellum	Decreased 1.7X week 2 and 4 pi Decreased 1.3X week 6 pi	RPA	[20••]
Bcl-X (L) mRNA	↑	Hippocampus Cerebellum:	Increased 1.8X at week 12 pi Increased 2.1X at week 12 pi	RPA	[20••]
Bax mRNA	-	Brain	At 4 weeks pi	RPA	[20••]

Abbreviations: ↑, increased; ↓, decreased; –, no change; HPLC, high-pressure liquid chromatography; pi, post infection; PND, post-natal day; RPA, RNAse protection assay.

response, other researchers suggested that more extensive sequence comparisons do not support the contamination hypothesis [47–49]. Some of the discussion revolves around the question 'how different is different enough to distinguish between human isolates and laboratory contaminants?', a question that has no real answer. A better understanding of the biology of BDV may help advance the field, as issues of crossreactivity of BDV reagents (such as animal strain reagents being used for searching for human infection) have not been well considered. There is also a lack of agreement on the type of study populations to be tested and on characterization and matching of subjects and controls. In addition, some human isolates have not undergone independent confirmation studies, thus failing to meet the scientific criterion of 'reproducibility' for general acceptance of results.

Conclusions

The field of BDV research and BD pathogenesis studies is a fascinating and expanding area. As more becomes known about the unique biology of this agent, the mechanisms behind those observations will provide valuable information for the scientific and medical communities. In an area in which multidisciplinary studies abound, BDV scientists have the opportunity to study interactions between the structure and function of the nervous system, including neuroanatomy, neurochemistry, neuroimmunology and neurodevelopment. Although modeling of human developmental diseases is difficult in animal systems, BDV infection of the neonatal rat represents the best characterized model for studying infection-based neurodevelopmental disease and, perhaps, for studying autism spectrum disorders. Future directions need to include a consensus on human BDV infections and disease outcomes, encourage scientists to work together to gain further acceptance of BDV's utility in animal model studies, and better appreciation of the multidisciplinary approach to BD pathogenesis research.

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- • of special interest
- •• of outstanding interest

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