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REVIEW ARTICLE

Borna Disease Virus and the Brain

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ABSTRACT: Viruses with the ability to establish persistent infection in the central nervous system (CNS) can induce progressive neurologic disorders associated with diverse pathological manifestations. Clinical, epidemiological, and virological evidence supports the hypothesis that viruses contribute to human mental diseases whose etiology remains elusive. Therefore, the investigation of the mechanisms whereby viruses persist in the CNS and disturb normal brain function represents an area of research relevant to clinical and basic neurosciences. Borna disease virus (BDV) causes CNS disease in several vertebrate species characterized by behavioral abnormalities. Based on its unique features, BDV represents the prototype of a new virus family. BDV provides an important model for the investigation of the mechanisms and consequences of viral persistence in the CNS. The BDV paradigm is amenable to study virus–cell interactions in the CNS that can lead to neurodevelopmental abnormalities, immune-mediated damage, as well as alterations in cell differentiated functions that affect brain homeostasis. Moreover, seroepidemiological data and recent molecular studies indicate that BDV is associated with certain neuropsychiatric diseases. The potential role of BDV and of other yet to be uncovered BDV-related viruses in human mental health provides additional impetus for the investigation of this novel neurotropic infectious agent. © 1997 Elsevier Science Inc.

KEY WORDS: Immunopathogenesis, Brain homeostasis, Viral persistence, Psychiatric disorders, Neurodegeneration, Behavior.

INTRODUCTION

To establish a persistent infection in its host, a virus has to meet two essential requirements. First, it must avoid clearance by the host immune surveillance. Second, it must adopt a noncytolytic strategy of multiplication. To achieve these goals, viruses have developed a plethora of strategies [1,52].

The central nervous system (CNS) has intrinsic properties that favor the establishment of viral persistence [116], namely: (a) the existence of the blood–brain barrier and the paucity of immune elements in the brain, that provide the CNS with an immunologically isolated environment; (b) viral replication is often restricted in cells of neuroectodermal origin, thus facilitating persistence; (c)

viral spread over long distances within the CNS is facilitated by the intricate networks of cell processes and cell interactions.

Evidence provided by epidemiological and clinical data, together with virological studies, have led to the hypothesis that chronic viral infections of the CNS contribute to human mental disorders of unknown etiology. It is therefore of interest to identify relevant infectious agents, as well as to understand the mechanisms by which viruses persist in the CNS and interfere with brain functions.

Borna disease virus causes CNS disease in a broad range of vertebrate animal species that is manifested by behavioral abnormalities and diverse pathology. BDV provides an important paradigm for the investigation of the mechanisms and consequences of viral persistence in the CNS. Studies on this viral system will contribute to the elucidation of immune-mediated pathological events involved in virally induced neurological disease, as well as the mechanisms whereby viruses induce neurobehavioral disturbances in the absence of the hallmarks of cytolysis and inflammation. Moreover, serological data and recent molecular epidemiological studies indicate that BDV can infect humans, and is possibly associated with certain neuropsychiatric disorders. Based on its unique biological and genetic characteristics, BDV represents the prototype of a new family of viruses. Consequently, investigations on BDV may provide new insights into virus–cell interactions in the CNS. The prospect of finding other BDV-like viruses, some of them possibly clinically relevant, provides further impetus for the study of this novel neurotropic infectious agent.

The purpose of this review is to discuss our present knowledge of BDV persistence in the CNS. We will first briefly describe the biology of BDV, with special focus on the recent developments regarding its molecular biology. We will then examine the interaction between BDV and the CNS. Finally, we will discuss the evidence supporting a possible role of BDV in certain human mental disorders.

BORNA DISEASE VIRUS

Early descriptions of Borna disease (BD) date back to the end of the eighteenth century [68]. BD was recognized as a sporadically occurring encephalopathy that affected horses and sheep in Southeastern Germany. The disease owes its name after the town

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of Borna, in Saxony, where many horses from a cavalry regiment died during an epidemic in 1895. Early studies showed that BD could be transmitted by using brain homogenates from diseased horses, demonstrating the infectious nature of the disease [101,125,155,188]. Sensitivity to detergents, ultraviolet light, and organic solvents, together with filtration studies suggested that BD was caused by an enveloped virus, the Borna disease virus.

Besides horses and sheep, infections with BDV have been documented in cattle, cats, donkeys, rabbits, and ostriches [23,35,128,130,172]. Sporadic cases of BD have also been reported in several other animal species [172]. Moreover, experimental infections have revealed the remarkable wide host range of BDV, which includes birds, rabbits, and rodents, as well as nonhuman primates [5,77,90,105,124,126,150,172,177,203,205]. BDV infection leads, after a variable incubation period, to diverse pathological manifestations that depend on the species, immune status and age of the host, route of infection, and the particular strain of virus used for infection. The adaptation of BDV to small laboratory animals, such as the rabbit and the rat, has facilitated more detailed studies on BDV pathogenesis and the neurobehavioral alterations accompanying the infection.

Filtration experiments estimated that BDV particles had a size of 80 to 125 nm [155]. Recent electron microscopy studies have shown that preparations of cell-free infectious BDV contain enveloped particles of roughly spherical morphology, approximately 100 nm in diameter [74,230]. Intracytoplasmic virus-like particles with similar characteristics have also been observed in thin sections of BDV-infected cells [42].

All BDV isolates are characterized by being highly neurotropic and by having a noncytolytic strategy of replication [87]. Cells and tissues of nonneural origin exhibit only a low susceptibility to infection. The paucity of cell-free virus associated with BDV infection has long hampered its molecular characterization. The establishment of persistently infected cell lines and the use of molecular genetic approaches has enabled the recent progress in the molecular characterization of this infectious agent. These studies have demonstrated that BDV is a nonsegmented, negative-stranded (NNS) RNA virus [43,50,123,216]. The cloning and complete sequencing of two BDV isolates has uncovered the genomic organization of BDV [31,44,48,183]. The genome is about 8.9 kb long, with complementary 3' and 5' untranslated regions at its termini (Fig. 1). The antigenomic polarity contains information for at least five open reading frames (ORF). Similarly to other members of the *Mononegavirales*, the genome can be divided in three main gene blocks: block 1 codes for the nucleoprotein and polymerase cofactors, represented by the BVp40 (ORF I) and BVp24 (ORF II) proteins of BDV; block 2 codes for the matrix and virus envelope proteins, whose likely counterparts in BDV are the BVp16 (ORF III) and BVp56 (ORF IV) proteins, respectively; and block 3 codes for the viral polymerase, identified as ORF V in the BDV genome.

The molecular biology of BDV has been the subject of recent reviews [30,48,183] and is not the focus of this review. We will only briefly describe here the main features that distinguish the replication and gene expression regulation of the BDV genome.

The BDV nucleoprotein (NP) BVp40 is present at high levels in infected cells and tissues. This protein is likely encoded in two isoforms of 38 and 40 kDa [81,94,165]. This may be related to the presence of two in-frame initiation codons in the BVp40 gene sequence. The BVp24 protein is acidic, with a high Ser/Thr content and is phosphorylated at serine residues [94,211,212]. These features are consistent with the phosphoprotein (P) transcriptional activator found in other NNS RNA viruses. The transcription unit encoding BVp24 can also direct the synthesis of a polypeptide of 10 kDa (BVp10). Recent data from our laboratory indicate that

BVp10 is present in infected cells. The ORF encoding BVp10 starts 46 nucleotides upstream from BVp24 and overlaps, in a different frame, with the 213 first nucleotides of ORF II (Fig. 1). The function of BVp10 is presently unknown. A similar situation has been described for the P gene of Sendai virus [46] and vesicular stomatitis virus [202]. BDV ORF III (BVp16) likely represents the BDV matrix (M) protein. In contrast to other NNS RNA viruses, BDV M protein is glycosylated and data suggest that it might be present at the surface of the virion envelope [111]. ORF IV is predicted to encode for a polypeptide of 56 kDa (BVp56). Sequence features suggest that this protein is a viral surface glycoprotein (GP). Recent reports have provided experimental evidence that BVp56 is involved in virus entry [74,185]. BVp56 is present as two forms in BDV-infected cells [74]. One form of approximately 84 kDa (GP-84) corresponds to the full-length product encoded by ORF IV and accumulates in the endoplasmic reticulum. The molecular weight of this polypeptide, higher than 56 kDa, is due to glycosylation. A shorter product of 43 kDa (GP-43) corresponds to the C-terminus of GP-84 and is generated via cleavage by the cellular protease furin [73]. Moreover, GP-43 is present at the surface of infected cells. Both GP-84 and GP-43 are associated with infectious virions. These features indicate a novel maturation pathway for a NNS RNA virus surface GP and, hence, for the assembly of BDV particles [74]. ORF V is capable of encoding a polypeptide with a predicted molecular mass of 180 kDa, whose deduced amino acid sequence displays strong homology with the NNS RNA viral polymerases (L protein family) [44]. This homology is particularly high in the case of the conserved putative catalytic domain.

BDV has the property, unique among known animal NNS RNA viruses, of a nuclear site for the replication and transcription of its genome [43]. Consistent with this finding, BDV ribonucleoproteins (RNP) are found in the nucleus of persistently infected cells [43]. As with other NNS RNA viruses, BDV RNP are infectious upon transfection of susceptible cells [43].

BDV exhibits a complex transcriptional pattern in infected cells. Subgenomic messenger RNAs (mRNAs) encoding BVp40 and BVp24 are monocistronic. In contrast, mRNAs encoding the M, GP, and L proteins are polycistronic. In the other NNS RNA viruses, these polypeptides are usually encoded by monocistronic mRNAs. In addition, BDV does not exhibit the configuration of transcription termination signal, intergenic region, and transcription initiation signal that is characteristically present at the gene boundaries of NNS RNA viruses [213]. BDV transcription units and transcriptive signals frequently overlap (Fig. 1). Moreover, BDV utilizes the host splicing machinery to generate some of its mRNAs [45,186].

Genomic stability is another striking characteristic of BDV. The two BDV complete genomic sequences yet determined have more than 95% homology at the nucleotide level [31,44]. This is remarkable for RNA virus isolates with different origin and passage history in animals and cultured cells. These sequences are also very similar to those obtained from field cases of BD in different animal species [21,184], as well as to sequences derived from human cases of BDV infection [29,49,108,180]. This extraordinary sequence conservation, uncommon for an animal NNS RNA virus, may indicate that there is a prevalent and stable species of BDV in nature, with the ability to infect several animal species, including humans. The reasons for the great stability of the BDV genome are unknown. Interestingly, sequence analysis reveals that the L proteins of BDV and *Sonchus yellow net virus*, a plant nucleorhabdovirus that also replicates in the nucleus, are the most distantly related to the L proteins from animal rhabdoviruses, raising intriguing questions about the evolutionary origins of BDV [44].

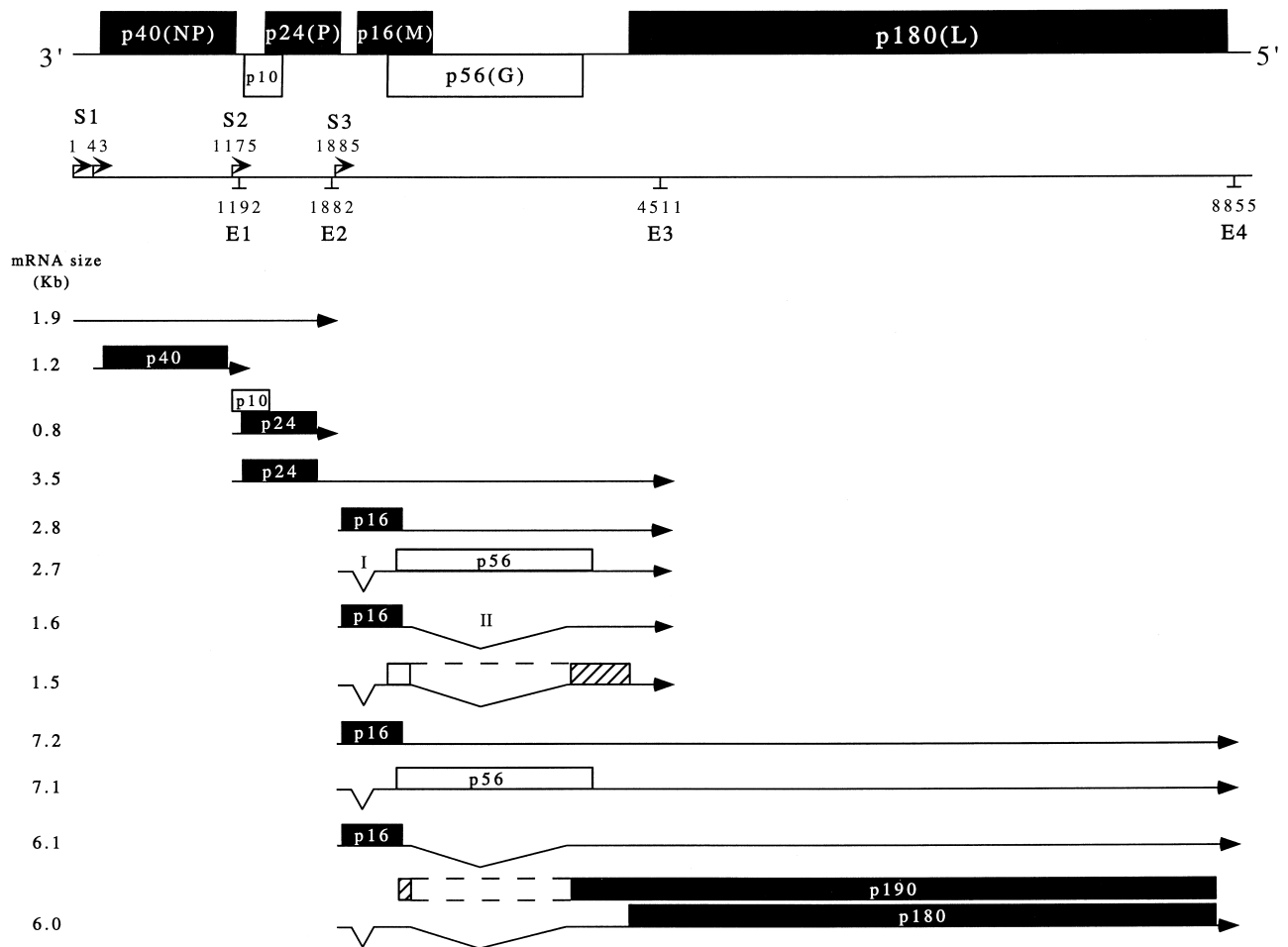


FIG. 1. Genomic organization and transcriptional map of BDV. BDV open reading frames are represented by boxes at the top. Different shades correspond to usage of different reading frames within the antigenomic polarity of the BDV genomic RNA. The location of transcription initiation and transcription termination sites are indicated by S and E, respectively. Positions of BDV introns I and II are indicated.

BDV is unique not only for its extraordinary wide host range, combined with a remarkable genomic stability, but also in various aspects of its biology. Expression of the BDV compact genome is regulated by an overlap of transcription units and transcriptive signals, an overlap of ORF, a readthrough of transcription termination signals and RNA splicing. The concurrent use of such a diversity of strategies for the regulation of gene expression is unique among known NNS RNA viruses. Taken altogether, this has led to the proposal that BDV represents the prototype of a new taxon of NNS RNA viruses [48,183].

BDV INFECTION OF THE CNS

Neuroinvasion and Propagation of BDV in the CNS

The portal of entry for BDV infection has not formally been identified. However, field observations have led to the hypothesis that a primary route of infection might be through the nose and the olfactory neuroepithelium [172]. Animals may become infected by contact with saliva, excretions, nasal secretions, or by exposure to contaminated food or water. Contact experiments have suggested that healthy carrier animals might represent a source of infection, because the disease can be transmitted between horses and sheep

living in the same stable [172]. Oral infection has been achieved in several animal species, suggesting that the virus could also be transmitted by the oral/gastric route [83,130]. Finally, although formal evidence is lacking, colostrum and milk could play a role in the infection of the newborn [172].

Experimental infection of the rat has allowed the dissection of the dissemination pathway of BDV [36,140]. After initial replication in the neurons located at the site of entry, BDV likely migrates intraaxonally in an anterograde or retrograde direction towards the CNS. The pathway of dissemination of BDV following intranasal inoculation of the rat has been thoroughly analyzed [140,190]. The virus initially replicates in the neuroreceptor cells of the olfactory epithelium. Subsequently, the virus gains access to the cells of the main olfactory bulb, including the tufted and mitral cells, as well as the periglomerular cells, the granule cells, and the short axon neurons of the internal granular layer of the main olfactory bulb. Following invasion of these sites, the virus will reach the efferent targets of the main olfactory bulb, like the anterior olfactory nucleus, the prepiriform cortex, the olfactory tubercle, the olfactory amygdala, and the entorhinal cortex. Further spread is then possible into numerous diencephalic and telencephalic areas, through polysynaptic neuronal connections. Likewise, experimen-

tal infection into the foot pad of a rat results in a retrograde transport of BDV, through the sciatic nerve, and then from neuron to neuron into the CNS [36]. Moreover, evidence of oral infection suggests that the nerve endings innervating intestinal regions are suitable for uptake of the virus and its transport to the CNS [130]. Alternatively, oral uptake of BDV may also result in the infection of the gut epithelium. The virus could then gain access to the surrounding lymphoid tissue, infect target blood cells, and be transported by the bloodstream into the CNS. The location of the initial site of entry will condition the incubation period preceding the onset of overt BD, reflecting the time required for viral transport into the brain.

A similar mode of dissemination has been described for rabies virus, another NNS RNA virus [76]. Both rabies and BDV spread along neuronal chains. The manner in which these chains are utilized is determined by the natural connections of neurons. Such a mode of spread is compatible with a synaptic transfer of these viruses. Moreover, the lack of detection of mature virus particles at any stage during BDV propagation has led to the hypothesis that viral spread ensues in the form of bare RNP [43,76]. This hypothesis has been further strengthened by the demonstration that RNP are infectious [43].

Within the CNS, BDV will exhibit a preferential tropism for the limbic system, including the hippocampus [37,78,140]. This area will eventually harbor the highest viral load regardless of the initial mode of infection, even if viral spread is not restricted to the limbic system and will diffuse throughout the CNS [78]. Hence, BDV is also found in the hypothalamus, thalamus, and cerebral cortex. Viral antigen accumulates in the nucleus, the perikaryon, and processes of the infected neurons [77]. The nucleolus, however, remains free of antigen. In the nuclei of infected neurons, aggregates of virus-specific material are likely to form the Joest-Degen inclusion bodies that characterize BDV-infected cells [100].

The topography of the infection in the hippocampus is particularly interesting. The stratified distribution of viral markers coincides with that of some excitatory amino acids receptors, in particular the glutamate receptors [78]. Also, specific neurotransmitter pathways are affected by BDV infection [122,198,200]. This could indicate that these receptors are used by the virus or by viral RNP for their transneuronal transfer. Besides neurons, glial cells are also permissive for BDV. Virus antigen and RNA appear rather early in astrocytes, followed by oligodendrocytes, ependymal cells, as well as Schwann cells in the peripheral nervous system [37]. It is probable that glial cells become secondarily infected after BDV release by the neurons. In agreement with this hypothesis, Purkinje cells are the only cells in the cerebellum containing BDV antigen early after infection. Later on, glial cells also become infected [8]. This is also reminiscent of findings described in other biphasic CNS diseases caused by RNA viruses, such as Theiler's virus infection of the mouse. In this case, the acute phase is characterized by an active viral replication in neurons. Subsequently, the virus accesses glial cells in the spinal cord, which is required for the establishment of persistence [97,99].

In the late stages of infection, BDV diffuses centrifugally, probably by using an anterograde axonal transport, and viral markers have been detected in peripheral nerves of all tissues and organs. Consequently, many tissues and organs become positive for viral markers [78,190,231]. These include lachrymal, salivary or sebaceous glands and, as a rule, any ectodermal/epithelial tissue that can be reached by BDV through central or peripheral innervation. However, it is likely that extra neural tissue will become eventually infected only if the virus is delivered through the peripheral axons for a long time.

The dissemination of BDV within the host could effectively

ensue in the form of RNP. However, horizontal transmission of BDV would likely require shedding of mature, enveloped particles with the ability to initiate infection in a new host. Despite numerous attempts, such particles have never been detected at any stage during BDV infection [76]. Therefore, the extremely low levels of infectious virus shed by infected animals may explain the poor rate of contagion of BDV.

Neuropathogenesis

Neuropathogenesis of the natural BDV infection. The neuropathogenesis of naturally occurring BD has been best characterized in the horse, where more than 80% mortality is observed. There is also increasing evidence that horses can be subclinically infected with BDV [7,84,117,146]. Borna disease is defined as a nonpurulent polioencephalomyelitis [77,101,188]. The inflammatory cells form massive perivascular cuffs, as well as more diffuse tissue infiltrates. Inflammatory infiltrates are present predominantly in the gray matter, but can also invade the underlying white matter. Reactive astrocytosis usually follows the acute phase of the disease. Interestingly, despite this strong inflammatory reaction, neuronal degeneration is limited. Horses may develop dimness of vision and partial or complete blindness, as a result of the degeneration of the optic nerve and neurons in the retina, likely caused by the lymphocytic infiltration [182,233]. However, BDV may also replicate in the retina without exerting any overt neuronal damage [84]. The brain regions most affected by the inflammatory response are the olfactory bulb, the hippocampus, and the caudate nucleus [102]. Inflammation is moderate in the spinal cord and absent in the cerebellum [101]. The colocalization of cell infiltrates with sites of high viral expression suggests that the inflammatory reaction is due to the presence of viral antigen.

The neuropathological features of BD in sheep are very similar to those described in horses [15,16,101]. Symptoms observed during natural BDV infection are summarized in Table 1.

Neuropathogenesis of the experimental BDV infection. A broad spectrum of animals can be experimentally infected with BDV (Table 1). The rat is the most commonly used model for the study of BDV pathogenesis. The age, immune status, and genetics of the rat, as well as viral factors can influence the course of infection [85,86,88,125,150,151].

Adult rats infected intracerebrally or intranasally with BDV usually develop an immune-mediated biphasic behavioral disease. Rats display clinical signs and a histopathological picture very similar to that described for the naturally infected horse [36,38,39,90,150,151]. The onset of clinical signs, including movement abnormalities, aggressive and hyperactive behavior, coincides with the appearance of an inflammatory reaction in the brain that reaches its maximum of severity between days 30 and 40 after infection. Parts of the limbic system, the cerebral cortex, and the olfactory bulb exhibit the strongest inflammatory reaction, which is limited to the gray matter. Some level of inflammation is also observed in the basal ganglia, the diencephalon, and to a lesser degree, in the midbrain and medulla. This extensive inflammatory reaction leads to neuronal destruction that in some cases may cause a hydrocephalus. As with the infection of horses, the cerebellum rarely shows signs of inflammation.

High virus replication is observed in the eyes of BDV-infected rats, which is accompanied by retinitis and leads to blindness due to the neuronal loss in the retina.

The initial aggressive hyperactive behavior is followed by apathy, somnolence, and depression. Eventually, some animals may also develop obesity [150]. This chronic phase is characterized by a steady decline in the inflammatory reaction, despite continuous high viral load in the CNS. To our knowledge, this has

TABLE 1
BEHAVIORAL ABNORMALITIES AND NEUROLOGICAL SYMPTOMS IN THE NATURAL AND EXPERIMENTAL BDV INFECTION

Species	Neurological Symptoms	Behavioral Abnormalities	Authors	References
Natural Infection				
Horses	a) meningitis, encephalitis, movement disorders, ocular disorders; late stages: paralysis	a) initial stages of disease: excitability or depression; later stages: severe depression, somnolence, apathy, anorexia	a) Zwick, 1939; Rott and Becht, 1995	[233,172]
	b) no symptoms reported	b) not reported	b) Lange et al., 1987; Herzog et al., 1994; Kao et al., 1993; Nakamura et al., 1995; Bahmani et al., 1996	[117,84,104,146,7]
Sheep	a) encephalitis; symptoms very similar to horses	a) symptoms similar to horses	a) Nicolau and Galloway, 1928; Ludwig, et al., 1988;	[155,125]
Cats	b) no symptoms reported	b) not reported	b) Matthias, 1954	[133]
	a) nonsuppurative encephalomyelitis ("staggering disease")	a) altered mentality, depression, anorexia	a) Lundgren and Ludwig, 1993; Novotny and Weissenböck, 1995	[128,156]
	b) no overt clinical symptoms	b) not reported	b) Lundgren et al., 1993; Nakamura et al., 1996	[127,145]
Experimental infection				
Hamster	not reported	not reported	Anzil et al., 1973	[5]
Mouse	subacute chronic encephalomyelitis in some strains	hyperactivity and aggressiveness (only in MRL/+ strain)	Kao et al., 1984; Rubin et al., 1993	[105,177]
Lewis rat	a) neonatal infection (i.c.*): no inflammation	a) learning disabilities, motor and emotional disturbances, abnormal ingestive behavior	a) Narayan et al., 1983a; 1983b; Dittrich et al., 1989; Bautista et al., 1994	[150,151,57,9]
	b) adult infection (i.c.) biphasic disease; meningoencephalomyelitis, retinitis, ataxia, priapism (males), followed by blindness, hydrocephalus;	b) acute phase: hyperactivity, aggressiveness, motor and behavioral alterations, ingestive disturbances, self-mutilation, later stages: apathy, depression, lack of self-grooming	b) Narayan et al., 1983a; 1983b; Hirano et al., 1983	[150,151,90]
Rabbits	acute encephalitis, retinitis, blindness, paralysis	depression, somnolence, inattention; anorexia	Zwick, 1939; Krey et al., 1979; Ludwig et al., 1988; Nicolau and Galloway, 1928	[233,114,125,155]
Tree shrews	mild encephalomyelitis 1) in some solitary kept animals; reduced muscle tonus and partial paralysis 2) paired animals	1) initial phase of hyperactivity, eating and sleeping disorders followed by a later hypoactive decline phase with reduced self-comfort behavior. 2) disturbed social and sexual behavior	Sprankel et al., 1978	[203]
Rhesus monkeys	acute encephalitis; retinitis without blindness, paralysis of hind limbs	apathy, anorexia, sometimes somnolence (i.c. infection); excited, disinhibited, aggressive (i.n. infection†)	Zwick, 1939; Stitz et al., 1980; Krey et al., 1982; Cervos-Navarro et al., 1981	[233,205,115,40]

* i.c. = intracerebral infection; † i.n. = intranasal infection.

not been observed in other viral infections. The mechanisms underlying this delayed immune-tolerance are unknown. It is possible that sustained expression of high levels of viral antigens may result in the exhaustion of high affinity virus-specific T-cells [143,217].

In contrast to the situation depicted above, BDV neonatal infection of the rat causes a life-lasting persistent infection that is characterized by the lack of a cellular immune response to BDV and the absence of clinical signs of BD [38]. This infection has

been designated as a "persistent, tolerant infection of the newborn" (PTI-NB) rat [38]. Nevertheless, the humoral response to BDV is not significantly impaired in PTI-NB rats [38,150]. Brains of PTI-NB rats harbor a viral load comparable to that found in rats with acute BD [36,88,150,207], illustrating the noncytolytic replication of BDV. Although PTI-NB rats do not show clinical signs, they exhibit distinct deficiencies in emotional and cognitive functions [9,57], as well as physiological and neurodevelopmental abnormalities [8,9]. Hence, the PTI-NB rat provides a valuable model to investigate the consequences of BDV infection in the CNS without immune-mediated damage (see sections 3.3 and 3.4).

BD as an immune-mediated neurological disease. Immunological mechanisms frequently contribute to the pathophysiology of virus-induced CNS diseases [1]. Experimental BD provides a valuable model to study T-cell-mediated immunopathology in the CNS [125,204].

The cell-mediated immune response to BDV plays an essential role in the development of BD. This has been shown by studies on immunocompromised animals. BDV infection of rats treated with cyclophosphamide, cyclosporin A, or of athymic rats does not lead to inflammation and development of disease [88,151,209]. However, adoptive transfer of spleen cells from BD rats into BDV-infected immunosuppressed recipients results in inflammatory changes in the brain and concomitant BD [151]. In contrast, transfer of immune serum does not lead to disease, demonstrating that antibodies are not involved in the immunopathological process induced by BDV [90]. Studies on PTI-NB rats provide further evidence for the role of the cell-mediated immune response in the development of BD. BDV replicates in the thymus of PTI-NB rats [176]. As with other viruses, early expression of viral antigens in the thymus can promote clonal deletion of T-cells that would normally respond to the infectious agent [96]. Thus, the lack of BDV-specific precursor T-cells in the spleen and lymph nodes likely explains the absence of an inflammatory reaction in the CNS of PTI-NB rats.

Pathogenic role of CD4⁺ and CD8⁺ T-cells in BD. Both CD4⁺ and CD8⁺ T-cells are present in the CNS cell infiltrates and contribute to the immune-mediated pathology associated with BD [19,204]. The role of CD4⁺ T-cells in BD was supported by the isolation of a BDV-specific CD4⁺ T-cell line with the ability to induce disease upon adoptive transfer into BDV-infected immunosuppressed recipients [167,169]. Adoptive transfer of these CD4⁺ T-cells into immunocompetent uninfected rats did not cause encephalitis or disease, demonstrating that this BDV-specific T-cell line was not encephalitogenic per se. Recent evidence indicates that a direct cytotoxic activity of CD4⁺ T-cells plays a rather limited, if any, role in BD [197]. Although BDV-specific CD4⁺ T-cells can exhibit major histocompatibility complex (MHC) class II restricted cytotoxic activity *in vitro* [167,168], there is not, however, convincing evidence of such an activity *in vivo* [162,197]. It should be noted that CD4⁺ T-cells may acquire cytotoxic activity during *in vitro* cultivation [65]. In addition, there is no evidence of MHC class II expression in neurons, the main cell type destroyed by the inflammatory response [38,167,206]. Furthermore, CD4⁺ T-cells are present predominantly in perivascular cuffs, whereas few reach the brain parenchyma, where the immune-mediated damage occurs [20,197]. Besides, adoptive transfer of noncytolytic virus-specific CD4⁺ cells into BDV-infected immunosuppressed recipients can lead to the development of BD only if CD8⁺ T-cells enter the brain [163]. Development of disease in these recipients can be prevented by treatment with monoclonal antibodies that specifically block CD8⁺ T-cell activity [163,208]. Conversely, treatment of BDV-infected rats with anti-CD4⁺ antibodies does not effectively prevent inflammation and tissue damage in the CNS [162].

Taken together, these findings suggest that CD4⁺ T-cells contribute to the pathogenesis of BD predominantly as T-helper cells [20,163,197]. Nevertheless, a potential lytic role of virus-specific CD4⁺ T-cells cannot be completely excluded [197]. In particular, astrocytes may express MHC class II antigens [53,162], thus being potential targets for CD4⁺ T-cells. CD4⁺ T-cells have been shown to play a role in measles- and coronavirus-induced encephalomyelitis [121,132]. However, these cases are considered as examples of virus-induced autoimmunity, illustrated by the ability of these cells to trigger an inflammatory reaction in the CNS of uninfected animals.

On the other hand, there is ample evidence that CD8⁺ T-cells act as effector cells in the immune-mediated pathological reaction observed in BD. Elimination, or functional blocking, of CD8⁺ T-cells prevents both BDV-induced neurological symptoms and histopathological changes in the brain [20,162,197,207,208]. In addition, lymphocytes isolated from rat brains during acute BD exhibit MHC class I-restricted cytotoxic T-lymphocyte (CTL) activity *in vitro* [162]. Adoptive transfer of these lymphocytes into immunosuppressed BDV-infected recipients results in the extravasation of CD8⁺ T-cells in the brain parenchyma, leading to immunopathological and clinical signs similar to experimental BD [197]. Therefore, neuronal damage seen in BD appears to be mediated by the cytotoxic activity of CD8⁺ T-cells present in the brain parenchyma of BDV-infected rats.

CD8⁺ CTL can destroy cells presenting virus-derived peptides in the context of MHC class I presentation [232]. Evidence suggests that MHC class I antigens are expressed at the surface of astrocytes and neurons in the CNS of BDV-infected rats, concomitantly with the peak of CD8⁺ T-cell infiltrate in the CNS and the onset of neuronal degeneration [20,37,206]. Under normal conditions, cells of the CNS express very low, if any, MHC antigens, but their expression can be upregulated during pathological situations [55,58]. It has been proposed that virally-infected neurons avoid destruction by CTL because of the lack of MHC class I expression [103,157]. However, synaptically silent neurons may express MHC class I molecules [154]. BDV is one of the rare cases in which it has been shown that a viral infection of the CNS can induce neuronal expression of MHC class I *in vivo* [20,55,69,70,162].

The molecular mechanisms responsible for BDV-induced MHC class I expression by neurons are presently unknown. It is possible that BDV infection of neurons directly causes upregulation of MHC class I expression. Alternatively, induction of neuronal MHC class I expression may be due to the action of cytokines secreted by inflammatory cells or virally-infected glial cells [54,55,113]. Certain cytokines, including interferon (IFN) γ or β and tumor necrosis factor α (TNF α) can upregulate MHC class I expression on neurons *in vitro* and to some extent *in vivo* [58,224]. Evidence suggests that BDV-infected astrocytes could secrete IFN α/β , which, in turn, could induce MHC class I expression in neurons [162].

Neurons in the CNS are terminally differentiated cells without the capacity of proliferation. Consequently, destruction of virally-infected neurons by the immune response can cause an irreversible loss of cells whose functions are required for normal brain performance. The existence of the blood-brain barrier significantly restricts the degree of immunological surveillance and the vigor of the immune response in the CNS. However, activated virus-specific CTL can access the brain parenchyma. BDV-mediated induction of MHC class I expression on neurons can then be detrimental for the host by allowing destruction by CTL. Furthermore, this BDV-specific CTL activity is unable to control the infection and viral load is not significantly decreased after regression of the immune response. Thus, the immune-mediated damage is likely to

have more devastating consequences than those directly due to the mere BDV persistence in neurons.

Role of cytokines in BDV pathogenesis. Cytokines and free radicals have recently gained attention as other mediators of immunopathology. There is increasing evidence that certain stimuli, including viral and bacterial infections, can upregulate cytokine expression in the CNS, resulting in tissue damage and neurological disease [34,136,171,225]. Along with activated lymphocytes and macrophages present in immune infiltrates, resident CNS cells like neurons, astrocytes, and microglia are potential producers of cytokines [171]. Proinflammatory cytokines including IL-1 α , TNF α , and IL-6 can cause neuronal damage [118,171]. Levels of IL-6, TNF α , and IL-1 α mRNAs are significantly increased in BD rat brains and their expression correlates with the degree of inflammation and severity of neurological signs [190]. Moreover, production of IFN γ by infiltrating CD8⁺ T-cells could induce MHC class II expression on astrocytes and microglia. This, in turn, would enhance CD4⁺ T-cell activity and contribute to the immunopathology of acute BD. It has also been proposed that the decline of inflammation during the chronic phase of BD might be mediated by cytokines such as IFN γ [190].

Free radicals such as nitric oxide, generated by the inducible nitric oxide synthase (iNOS), can also cause direct neuronal damage [112,153]. Increased CNS expression of iNOS mRNA is observed in rats with BD. Levels of iNOS mRNA correlate with the severity of neurological signs and degree of inflammatory lesions [2,229]. The finding of up to 30-fold increased amounts of nitric oxide in BDV-infected rat brains suggests a possible contribution of nitric oxide to BD pathogenesis [92]. However, it is also possible that the iNOS mRNA expression in the CNS merely reflects a nonspecific activation of microglia, thus being more of a secondary than a primary pathogenic importance.

Disturbances in the Postnatal Maturation of the Cerebellum and Hippocampus

BDV is considered as a noncytolytic virus *in vitro*, because infected cultured cells do not exhibit impaired growth or survival [87]. Analysis of the situation in animals with BD is complicated by the massive inflammatory response accompanying the infection. Thus, it is difficult to distinguish cell and tissue damage directly caused by BDV replication from the damage due to the antiviral immune response. The PTI-NB rat model (see above) has provided a system to study virus-induced structural and functional CNS alterations without inflammation [38]. Studies on PTI-NB rats have suggested that BDV can induce a selective damage on specific neuronal populations [9]. However, this restricted neuronal degeneration is not considered as a "classical" cytopathic effect, but is rather due to an interference of BDV infection with the postnatal development of CNS areas that experience extensive postnatal maturation.

One of the most noticeable morphological features of the PTI-NB rat brain is the considerable cerebellar hypoplasia induced by BDV perinatal infection. Also, the dentate gyrus in the hippocampus undergoes progressive degeneration. These traits are accompanied by a prominent and widespread astrocytosis [204], defined as an increase in the number and size of cells expressing the glial fibrillary acidic protein (GFAP), an astrocyte-specific marker [60] (see also the increased levels of GFAP mRNA expression in PTI-NB rats shown in Fig. 2).

Other perinatal virus infections, including lymphocytic choriomeningitis virus (LCMV), rat parvovirus and reovirus type III, also induce damage to the cerebellum [139,158,166]. The cerebellum developmental arrest has been attributed in these cases to either an immune-mediated, or a virus-induced lysis of the dividing imma-

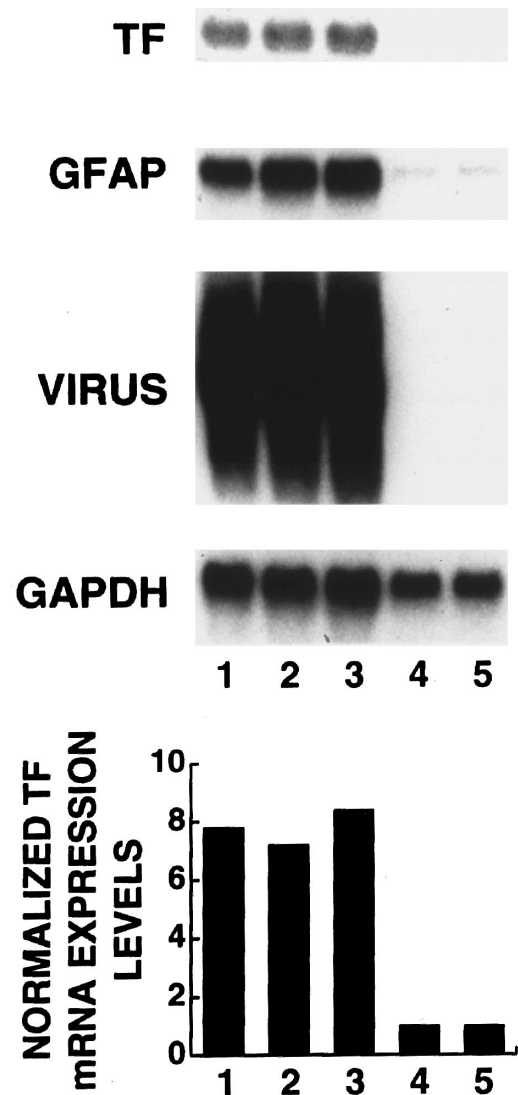


FIG. 2. Tissue Factor (TF) messenger RNA levels are markedly increased in the CNS of BDV persistently infected (PTI-NB) rats. Total RNA was isolated from brains of PTI-NB rats (lanes 1 to 3) and sham infected control rats (lanes 4 and 5), and analyzed by Northern blot hybridization for the expression of TF and glial fibrillary acidic protein (GFAP). Viral RNA was detected in BDV-infected rats using a BVp40 cDNA probe. Levels of TF mRNA were normalized to those of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). See also [75].

ture cerebellar granule cells. The characteristics of BDV-induced cerebellar hypoplasia have been detailed in a recent report [8]. The arrest of cerebellar development occurs between 7 and 14 days postinfection. This coincides with a progressive atrophy of the granule cell layers. In contrast to the other viral systems mentioned above, the granule cells are not infected by BDV. Purkinje cells are the predominant, if not the only, cell type infected in the cerebellum at the early stages of its development [8]. Therefore, it appears that BDV-induced cerebellar damage is caused by different mechanisms than those proposed with other perinatal virus infections. Evidence indicates that Purkinje cells play a main role in supporting the multiplication, maturation, and migration of the granular cells [195]. This role could be altered by BDV infection. The

massive astrocytosis observed in PTI-NB rats might also be involved in the cessation of granule cell migration, especially given the key role of astrocytes in providing the physical track upon which the granule cells migrate [82], as well as neurotrophic support to surrounding cells [60].

The hippocampus consistently harbors the highest viral load in PTI-NB rat brains [37,78,140]. Despite considerable replication of BDV in this area, there is little effect on the lifespan of the infected neurons. One notable exception is the dentate gyrus (DG), whose neurons are progressively destroyed [38]. The DG is formed primarily during the postnatal period. Although birth of cells in the DG begins embryonically, the vast majority (>85%) of DG granule neurons are generated after birth [79]. Hence, neuronal multiplication and migration will occur in this area well into adulthood. This represents a unique characteristic for neurons of the mammalian adult brain, that are usually considered as a whole as postmitotically differentiated cells [79].

Dentate gyrus degeneration has also been observed following persistent neonatal infection with LCMV [160]. It has been shown that degeneration of the dentate gyrus and abnormalities in synaptic function occur after LCMV clearance from the granule cells. Electrophysiological measurements demonstrated that these cells exhibit a persistent hyperexcitability. This was due to a decreased inhibition, usually mediated by a subpopulation of GABA interneurons that were affected by the infection [160].

The mechanisms underlying BDV-induced DG degeneration are presently unknown. Given the noncytolytic effect of BDV on other neuronal populations, one could hypothesize that BDV-mediated degeneration of DG granule neurons is likely due to either (a) the unique characteristics of these mitotically active neurons, or (b) the disruption of pathway(s) essential for DG maturation [214]. These questions warrant further investigations, especially considering the importance of the DG and hippocampus in learning and memory. In this respect, BDV represents an important model to investigate the pathogenesis of CNS disease linked to abnormal CNS development and to better understand the regulation of CNS plasticity.

BDV and Brain Homeostasis

The effector mechanisms involved in BDV-induced neuronal damage are still poorly understood. However, BDV infection is not likely to cause direct neuronal destruction but triggers instead a series of secondary events leading to cell damage within the CNS. Most of the CNS alterations induced by BDV infection of immunocompetent adult rats can be attributed to the virus-induced cellular immune response. However, BDV-mediated impairment of brain activity can also occur without the hallmarks of inflammation and widespread cytolysis, owing to the virus' ability to cause changes in neuronal organization and to interfere with brain cell functions required to maintain CNS homeostasis. The PTI-NB rat offers a unique system to study the mechanisms underlying these virus-CNS cell interactions.

Astrocyte functions are essential to neurons [60]. It is, therefore important to investigate the consequences on brain function associated with the prominent astrocytosis that characterizes PTI-NB rat brains. We have already mentioned the key role of astrocytes in providing a substrate for neuronal migration during brain development. The astrocytic network participates in brain information processing in cooperation with neuronal networks [47]. Astrocytes also play roles in the elimination of neurotoxins and in the production of various cytokines, which can act as neuronal differentiation factors [159]. Cytokines can contribute to the regulation of neuronal neurotransmitter gene expression and, hence, can dramatically alter synaptic function [171,225]. These changes can, in

turn, contribute to complex animal behavior as well as to plasticity processes accompanying learning and memory.

Expression of high levels of proinflammatory cytokines [190] and of complement cascade components [56] have been documented in the rat CNS during acute BD (see above). These molecules were likely to have been produced by inflammatory cells invading the brain parenchyma. Therefore, it was difficult to assess the intrinsic response of resident CNS cells. To separate these two events, Morimoto et al. used dexamethasone treatment of rats after BDV infection [141]. This synthetic glucocorticoid effectively prevented inflammation in the CNS of BDV-infected rats. However, several proinflammatory cytokines such as TNF α and IL-6 were still induced in the brains of dexamethasone-treated rats, suggesting that these might have been produced by CNS resident cells. However, dexamethasone treatment of rats per se may have a profound action on the cytokine expression pattern of CNS cells [181]. Studies on PTI-NB rats may provide valuable information regarding the contribution of CNS resident cells to disturbances in cytokine gene expression caused by BDV.

Perinatal BDV infection of the rat provides a system to dissect the elements involved in BDV-induced alterations of CNS homeostasis without inflammation. So far, very few studies have addressed such questions in this model. Recently, a BDV-induced upregulation of tissue factor (TF) was demonstrated in the CNS of PTI-NB rats [75]. TF, a transmembrane receptor, is the primary initiator of the coagulation protease cascade that results in the generation of the protease thrombin [62]. Besides, TF appears to have pleiotropic roles and has been implicated in signal transduction, angiogenesis and brain function [33,61,175,228]. In the CNS, TF is primarily, if not solely, produced by astrocytes [59]. TF expression is markedly increased during persistent infection with BDV, both *in vivo* and *in vitro* [75] (Fig. 2). This upregulation is due to BDV infection of astrocytes, and is mediated by an increased transcription of the TF gene and a stabilization of TF mRNA. The significance of this finding is still a matter of speculation. However, because this phenomenon occurs without hemorrhage, inflammation, or disruption of the blood-brain barrier, it raises interesting questions about the consequences of TF upregulation and suggests that TF may fulfill functions other than hemostasis in the CNS. There is increasing evidence that proteins of the coagulation and fibrinolysis systems may function in the CNS independent from blood clotting, such as regulating normal brain development and defending the brain against damage caused by stroke, trauma, and other injuries [131]. Any imbalance in the protease activities may contribute to neuronal damage. TF is the primary initiator of the thrombin activation system, a protease that has been implicated in several neurodegenerative diseases, such as Alzheimer's disease [131,135]. Interestingly, thrombin is predominantly expressed by dopaminergic neurons of the mesencephalon [221]. Therefore, this may suggest a link between TF upregulation and the dopaminergic abnormalities observed in BD rats (see section 3.5). These findings suggest that upregulation of TF expression due to BDV infection may play an important role in the CNS response to this viral assault and perhaps also in the BDV-mediated disturbances of CNS function. Whether CNS expression of other genes, including cytokines, is specifically altered by BDV infection remains to be established.

Behavioral Disturbances Associated with BDV Infection

Behavioral abnormalities caused by BDV infection (Table 1) were first described in naturally infected horses and sheep [125]. The acute phase of disease is characterized by movement disorders, accompanied by excitability and hyperactivity. The later stages of the disease are frequently manifested by somnolence,

depression, apathy, and anorexia. Mortality rates are as high as 80–100% in horses and 50% in sheep. Animals that survive remain chronically infected and recurrent episodes with exacerbation of behavioral alterations may occur [83].

Both host and viral factors influence significantly the behavioral abnormalities associated with BDV infection. For example, hamsters and black-hooded rats do not develop apparent behavioral or neurological symptoms, despite the presence of virus in the CNS [5,85]. Strain specific differences in the susceptibility to disease have also been observed in mice. Thus, although BDV induces encephalitis to a similar degree in MRL/+ and MRL/lpr mice, only MRL/+ mice develop behavioral abnormalities [177]. This dissociation between inflammation and symptoms indicating the onset of the disease may reflect differences in the composition and immunological properties of the cellular infiltrate [177]. The host genetic background likely contributes to these differences. Alternatively, host factors could also modulate the vulnerability of brain cells to the inflammatory reaction [78].

BDV-infected tree shrews illustrate important aspects of the consequences that BDV persistent infection may have in the behavior of highly developed animals [203]. Tree shrews (*Tupaia glis*) are phylogenetically classified at the root of the primates and exhibit a complex behavioral repertoire [201]. Tree shrews inoculated with BDV develop a persistent infection that is frequently associated with behavioral disorders. Interestingly, housing conditions have a strong influence on the behavioral manifestations after infection. Only a small number of animals kept in isolation show behavioral abnormalities, whereas all paired animals display profound sociosexual behavioral disturbances. These include a tendency to accept partners more quickly, a need for increased body contact, as well as a reversal in the role of the sex partners and an impaired breeding behavior of the females. Some animals kept in isolation also show neurological symptoms (Table 1). In these cases, an initial hyperactive phase, that coincides with the peak of the neurological symptoms, is followed by a phase of decline. All these animals recover, but relapse of clinical symptoms can occur in some cases. It is unknown why clinical symptoms are observed only in some animals, especially because all have comparable viral load in the brain and similar titers of serum antibodies to BDV [203]. The behavioral alterations exhibited by BDV-infected tree shrews are controlled by the limbic system and can be summarized as a disinhibition toward the environment [203]. Interestingly, evidence indicates that virus-specific lesions in neurons of the limbic system represent a hallmark of BDV infection.

Behavioral disturbances associated with BDV infection have been thoroughly studied in the rat model. Intracerebral inoculation of the adult immunocompetent rat causes a persistent infection that is associated with the previously described immune-mediated biphasic neurobehavioral disease (see section 3.2.2). BD rats develop a progressive movement and behavior disorder, accompanied by hyperactivity. Several studies have focused on the pharmacology and neurochemistry of this hyperactive syndrome [198,199,200]. Based on similarities with the behavioral disturbances caused in rats by neurotoxic or electrolytic lesions in the frontal cortex or its catecholamine afferents [215], it has been proposed that BDV-induced disturbances might be due to alterations of the dopamine system. Dopaminergic activity is enhanced in the prefrontal cortex of BD rats, revealed by high levels of 3,4-dihydroxyphenylacetic acid, the primary dopamine metabolite. Yet, dopamine receptor density is not affected in the prefrontal cortex [199]. Analysis of dopamine receptor density in rostral striatal areas, such as the nucleus accumbens, revealed a loss of dopamine reuptake sites correlated with decreased numbers of D₂ and D₃ receptors binding sites [200]. The nucleus accumbens is a

primary site of integration between limbic and motor information and has been involved in the control of motor, cognitive, emotional, and reward processes [119]. Thus, abnormalities in the mesocorticolimbic dopaminergic network may constitute the neural substrate of hyperactivity in BD [200]. Furthermore, similarities between these alterations and those observed in manic depressive or schizophrenic patients [161,187,223] may be an important pathophysiologic feature linking the BD rat model to human psychiatric diseases. Besides dopamine, alterations in other neurotransmitter systems have also been described in BD rats and include changes in mRNA levels of cholecystokinin, glutamic acid decarboxylase, and somatostatin [122].

The behavioral abnormalities observed in BD rats are likely to be a consequence of the inflammatory response. In particular, the high levels of cytokines produced by the immune infiltrates during the acute phase of disease could cause behavioral changes, including hyperactivity and aggressiveness [34,171]. The correlation of cytokine levels in BD rat brains with the degree of inflammatory reaction and severity of neurological symptoms supports this hypothesis. It should be reminded that the hippocampus frequently harbors a high viral load [37,78,140], thus becoming a primary target for the virus-specific inflammatory reaction. This brain structure plays essential roles in governing emotion, learning and memory [10]. Therefore, cytokine-mediated disturbances of hippocampal functions can cause severe behavioral abnormalities [17,89].

The later phase of BD is characterized by the decline of the inflammatory reaction and by symptoms like apathy and somnolence. This likely reflects the neuronal damage caused by the immune response.

In contrast to BDV-infected immunocompetent adult rats, PTI-NB rats develop a chronic infection with no overt signs of classic BD. However, PTI-NB rats exhibit distinct cognitive, behavioral and physiological abnormalities (Table 1) [9,57]. PTI-NB rats show impaired cognitive functions in different learning paradigms, including spatial discrimination and discriminated avoidance tasks [9,57]. Results from open field and neophobia tests revealed emotional disturbances in PTI-NB rats, manifested as a reduced resting behavior and decreased anxiety. Moreover, these animals displayed hyperactivity when placed in a novel environment [9]. Reduced body weight and length were also observed in PTI-NB rats, compared to age- and sex-matched uninfected controls. Impaired growth did not seem to be related to disturbances in growth hormone physiology, because levels of growth hormone, insulin growth factor type 1, and glucose were unchanged in PTI-NB rats [9]. These differences in growth appeared only after weaning, suggesting that the feeding behavior might have been affected by perinatal BDV infection. In addition, PTI-NB rats exhibited an altered preference for a saline solution when it was paired with either water, saccharin, or quinine solutions [9]. The mechanisms underlying this altered salt taste preference are unknown. Disturbances in the postnatal maturation of the cerebellum and hippocampus induced by BDV, which have been reviewed above, probably contribute to the neurobehavioral abnormalities observed in these animals.

BDV IN HUMANS

Clinical, epidemiological, and virological data indicate that viruses can persist in the CNS and induce progressive neurological disorders, which are associated with diverse pathological manifestations. These include alterations in behavior and cognition [138,142,227]. These findings have led to the hypothesis that viruses can contribute to neuropsychiatric disorders whose etiology remains unknown [227]. The wide host range of BDV, to-

gether with the observation that BDV-infected animals exhibit behavioral disturbances resembling some human mental disorders, prompted studies aimed at determining an association of BDV with these disorders.

Evidence of BDV Infection in Humans

Seroepidemiological studies. Seroepidemiological studies conducted in different laboratories over the last 10 years have consistently shown an increased BDV seroprevalence in neuropsychiatric patients (Table 2). However, considerable variations in prevalence rates have been described, ranging from 1.6 to 30% in psychiatric patients and 0 to 3.5% in controls (Table 2). Moreover, follow-up studies on psychiatric patients revealed a high BDV seroprevalence (21.1%), reaching up to 37% among patients with major depression [25]. Increased BDV seroprevalence has also been reported in immunosuppressed HIV-infected patients from Europe and Thailand [6,26], individuals with parasitic infections in Africa [26], as well as patients with neurological disorders [13].

These serological findings should be cautiously evaluated because of the following reasons: (a) In many cases, only limited information was provided about the composition of the subject group analyzed. Thus, factors such as the geographic area, the heterogeneity of diagnoses, and clinical status of patients may have significantly influenced these results. For example, a slightly higher percentage of seropositives is found in patients' sera derived from areas in southern Germany, where BDV is endemic in animals [12,14,173] (Table 2); (b) Differences in the experimental procedures used to detect antibodies to BDV in human sera may also have affected the results. In particular, the immunofluorescence assay used in many of these studies [25–27] is considered to be unreliable [110,218]. The use of immunofluorescence assays for BDV serology poses difficulties because of the very low BDV antibody titers in human sera [22]. Furthermore, sera from neuropsychiatric patients often contain autoantibodies that react with nuclear structures [67,120,194]. Thus, the nuclear localization of BDV antigens in infected cells makes necessary the use of appropriate controls to discriminate between BDV-specific staining and nuclear staining due to autoantibodies. Increased specificity and sensitivity are now provided by Western blot assays that use either BDV-infected cell extracts [66,218], or recombinantly expressed BDV antigens [108,180]. Interestingly, results from studies conducted using this improved serologic test have still shown a significantly higher seroprevalence in neuropsychiatric patients (6.5–30%) compared to nonpsychiatric control groups (0–1.4%) [66,108,180,218].

Detection of BDV antigens and RNA in human peripheral blood mononuclear cells. The cloning and molecular characterization of BDV [31,44] has allowed the development of specific and sensitive procedures for the detection of BDV RNA in biological samples. Using reverse transcriptase-PCR (RT-PCR) procedures, BDV RNA can be detected in the peripheral blood mononuclear cells (PBMC) of infected rats [176,193]. This finding led to the use of a similar approach to examine the prevalence of BDV in humans. Thus, BDV RNA was first detected in the PBMC from four out of six hospitalized psychiatric patients (inpatients) [29]. This initial study has been followed by more comprehensive molecular epidemiological investigations. BDV RNA prevalence of 37% (22 of 60) [108], or 10.9% (6 of 55) [95], has been documented in PBMC of Japanese neuropsychiatric inpatients. Studies on Japanese healthy blood donors revealed a viral prevalence of 4.6% (8 of 172) [108] and 0% (0 of 36) [95]. Studies on German neuropsychiatric patients revealed a BDV RNA prevalence in PBMC of 50% (13 of 26), whereas 0% (0 of 23) of normal controls were positive for viral RNA [180]. These studies have

also shown that patients harboring detectable levels of viral RNA in their PBMC are frequently BDV seronegative; conversely, BDV-seropositive individuals have frequently nondetectable levels of viral RNA [29,108,180].

Recently, a high BDV seroprevalence and viral RNA in PBMC have been detected in blood samples from Japanese patients diagnosed with chronic fatigue syndrome (CFS) [109,148]. BDV antibodies were detected in 33.7% (30 of 89) CFS patients, but only in three cases of 100 healthy controls. BDV RNA was detected in 16% (10 of 62) CFS cases and 4.6% (8 of 172) control cases. Patients with CFS frequently exhibit neuropsychiatric symptoms, including severe depression. The etiology of CFS is unknown, but evidence suggests the involvement of viral infections [93]. Thus, these preliminary and intriguing results deserve further investigation.

The number and nature of the infected cells in PBMC are still controversial. In persistently infected rats, the prevalence of BDV-infected cells in PBMC has been estimated at 1 per 5×10^6 cells [176]. The need of highly sensitive RT-PCR procedures to detect BDV RNA in PBMC from humans or BDV-infected animals indicates an extremely low viral load in PBMC. However, some investigators have reported that 10 to 17% of the monocytes of BDV-positive patients express BDV antigens [28,29]. This would suggest that about 1% of the PBMC are infected with BDV. In this case, even assuming a very low viral load per cell, detection of BDV RNA should not require the use of nested RT-PCR. The reasons for this apparent discrepancy remain to be determined. Data from the rat model suggest that BDV is present in a very small fraction of circulating stromal cell precursors, rather than in the monocytes [176]. PBMC could have become infected during passage through the brain, or they may represent primary targets with the ability to spread the infection into the CNS, a situation that has been proposed for other neurotropic viruses [64,149]. Intravenous injection of rats with cell-free BDV is not followed by the establishment of a persistent infection [36]. This argues against cells within the PBMC being primary targets for BDV.

Sequence analysis revealed a high degree of conservation of both inter- and intrapatient BDV sequences, as well as a close genetic relationship between human and animal-derived BDV sequences [29,49,180]. This finding is consistent with the remarkable genetic stability of BDV in animals, which has been dealt with in a previous section. However, a higher sequence variability has been reported in human BDV sequences derived from Japanese patients' PBMC [106]. This might reflect strain differences due to geographical location. Nevertheless, this higher variability may have been overestimated by the experimental procedures used to determine these sequences (see discussion in [180]).

The high genetic stability of BDV is an uncommon finding for an RNA virus. Because the mutation frequencies of RNA viruses exceed by more than a million fold those of their eukaryotic hosts, extremely rapid virus evolution is anticipated and frequently observed [91]. However, RNA viruses can also display long-term stasis both in nature and in laboratory conditions, as a result of selection for fit master sequences in rather constant environments [91,219].

The high sensitivity of nested RT-PCR allows the detection of very low levels of target sequences. This method is also prone to artifacts due to inadvertent contaminations with laboratory sources of BDV. Moreover, the high degree of BDV sequence conservation proscribes the use of sequence analysis to pinpoint cases of contamination. These concerns can be addressed by following well-established guidelines to avoid contamination problems during RT-PCR assays [137,180]. Nevertheless, the low level of viral RNA in PBMC, close to the sensitivity threshold of nested RT-PCR, can hamper the reproducibility of results between laborato-

TABLE 2
PREVALENCE OF BDV IN HUMANS

Subject Group	Test Parameter*	Geographic Area	Subject Specification	Subject Number	Percentage of Positives	Authors	References
Mental disorders							
	IF	USA	major depressive disorder (mainly outpatients‡)	265	4.5%	Amsterdam et al., 1985	[4,174]
	IF	Germany	healthy volunteers	105	0%	Rott et al., 1985	
	IF	Germany	inpatients with various psychiatric disturbances	694	0.6%	Rott et al., 1985	[174]
	IF	Germany	healthy volunteers	95	0%		
	IF	Southern Germany†	inpatients with various psychiatric diseases	1003	6.8%	Bechter et al., 1987	[12]
	IF	USA	surgical patients	133	3.5%		
	IF	USA	major depression (outpatients)	642	2.0%	Bode et al., 1988	[27]
	IF	USA, Germany	volunteers, blood donors, HIV negative patients	540	2.0%		
	IF	USA, Southern Germany†, Japan	psychiatric and neurological inpatients	5000	4-7%	Rott et al., 1991	[173]
	IF	Southern Germany†	control hospital patients	1000	1%		
	IF	Southern Germany†	psychiatric inpatients	2377	5.9%	Bechter et al., 1992	[13]
	IF/IP	USA	surgery patients	569	3.5%		
	IF/IP	USA	Major depression (unipolar and bipolar) surgery patients	550	2.2%	Bode et al., 1992	[26]
	IF	Germany	acute psychiatric inpatients (follow-up study)	365	2.2%		
	IF	Germany	acute psychiatric inpatients (follow-up study)	71	19.7%	Bode et al., 1993	[25]
	WB	USA	major depression (unipolar and bipolar)	138	6.5%	Fu et al., 1993	[66]
	WB	USA	healthy controls and nonpsychiatric patients	117	0.85%		
	WB	USA	schizophrenic outpatients	90	14.4%	Waltrip et al., 1995	[218]
	WB	Japan	normal control subjects	20	0%		
	WB	Japan	psychiatric patients	60	30%	Kishi et al., 1995b	[108]
	WB	Germany	inpatients with various psychiatric disorders	416	9.6%	Sauder et al., 1996	[180]
	nested RT-PCR	Germany	surgery patients	203	1.4%		
	nested RT-PCR	Germany	acute and chronic inpatients	6	66%	Bode et al., 1995	[29]
	nested RT-PCR	Germany	healthy blood donors	10	0%		
	nested RT-PCR	Japan	psychiatric inpatients	60	37%	Kishi et al., 1995a; 1995b	[107,108]
	nested RT-PCR	Japan	healthy blood donors	172	4.6%		
	nested RT-PCR	Japan	psychiatric inpatients	55	10.9%	Igata-Yi et al., 1996	[95]
	nested RT-PCR	Japan	healthy blood donors	36	0%		
	nested RT-PCR	Germany	psychiatric inpatients	26	50%	Sauder et al., 1996	[180]
	nested RT-PCR	Germany	healthy volunteers	23	0%		
Diseases associated with immune-suppression							
1) HIV-1 infection							
	IF	Germany	HIV infected individuals	460	7.8%	Bode et al., 1988	[27]
	IF	Germany	HIV antibody negatives	125	1.6%		
	IF/IP	Europe	HIV negative drug abusers	106	3.8%		
	IF/IP	Europe	asymptomatic HIV infection	1024	7.1%	Bode et al., 1992	[26]
	IF/IP	Europe	HIV infection (lymphadenopathy)	244	13.9%		
	ELISA	Thailand	HIV negative blood donors	118	2.5%		
	ELISA	Thailand	asymptomatic HIV infection	60	15%	Auwanit et al., 1996	[6]
	ELISA	Thailand	patients with AIDS	67	17.9%		
	ELISA	Thailand	HIV negative blood donors	103	1.9%		
2) parasitic infections							
	IF	East Africa	schistosomiasis and malaria	193	9.8%	Bode et al., 1992	[26]

* Assay system used to determine BDV prevalence. IF: immunofluorescence; IP: immunoprecipitation; RT-PCR: reverse transcriptase-PCR; WB: Western blot.

† These areas have been recognized as endemic for BD in animals.

‡ Inpatients and outpatients stand for hospitalized and nonhospitalized patients, respectively.

ries [179]. It is worth noting that although the detection of BDV RNA in PBMC provides a valuable diagnostic tool, it does not necessarily reflect the viral load in the CNS. For example, PTI-NB rats harbor extremely low levels of BDV RNA in their PBMC,

whereas high levels of viral antigen and RNA are expressed in the CNS [176,193].

Isolation of human BDV. Recently, BDV has been isolated from PBMC of three German psychiatric inpatients with affective

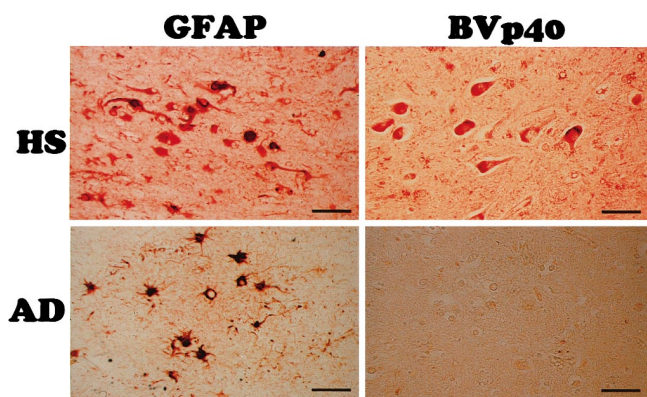


FIG. 3. Immunocytochemical detection of BVp40 antigen in the human CNS. Serial hippocampal sections of representative examples of one hippocampus sclerosis (HS) case and one Alzheimer's Disease (AD) case were analyzed for the expression of BVp40 antigen and glial fibrillary acidic protein (GFAP). Both HS and AD cases exhibited a prominent astrocytosis as determined by GFAP expression. BVp40 antigen was detected in the HS case but not in the AD case. See also [51]. Scale bar: 40 μ m.

disorders [24]. The isolation was achieved by cocultivation of PBMC with a human oligodendroglia cell line, followed by serial passages of these cells. Rescue of human BDV required the use of PBMC samples with strong viral antigen expression and at least 11 passages of the initial cocultivation [24]. These requirements to achieve virus isolation may explain why initial attempts to isolate BDV from humans had been unsuccessful [173]. Sequence analysis of the three human BDV isolates revealed a very close relationship with known BDV animal strains [49]. This high sequence conservation, together with the number of passages necessary to isolate the virus, has raised concerns about possible contamination with laboratory strains and stresses the need of further studies to confirm these findings.

Detection of BDV antigens and RNA in the human brain. Given the hypothesis that BDV contributes to human mental disorders, it should be expected that BDV also infects the human CNS. Some investigators have used RT-PCR to search for BDV sequences in autopsy brain material from cases of schizophrenia and affective disorders [189,192]. These initial studies failed to detect BDV-positive samples. However, a recent study has documented the detection of BDV antigen and RNA in human autopsy brain samples [51] (Fig. 3). The initial selection of the cases analyzed in this study was based on histopathological features resembling those observed in chronically BDV-infected animals. In particular, damage to the hippocampus and astrocytosis were used as screening criteria. Among 600 autopsy cases examined, five were found with sclerosis of the hippocampus and astrocytosis as the main histopathological findings. These patients presented with a clinical history of mental disorders manifested as severe depression and memory loss. In four of these five patients, immunohistochemistry and *in situ* hybridization revealed the presence of viral antigen and RNA in neurons and astrocytes within the hippocampal formation. In contrast, BDV markers were not found in control cases [51]. Detection of viral RNA required the use of nested RT-PCR, suggesting that failure to detect BDV RNA in previous studies, using single round RT-PCR, might have been related to lack of sensitivity. These findings demonstrate that BDV can persist in the human CNS, providing the basis for more comprehensive studies aimed at determining whether BDV persistence in the CNS is associated with specific human mental disorders.

BDV RNA has also been detected recently in clinical samples of brain tumors [147]. Viral RNA was detected in 5 of 16 specimens with grade 4 glioblastomas, but in none of 21 lower grade astrocytoma specimens. Patients with malignant brain tumors, especially glioblastoma multiforme, are frequently severely immunosuppressed [170]. These findings provide additional support to a possible relationship between immunosuppression and levels of BDV expression. Further studies are required to clarify this issue.

A recent report [177b] has described the detection of BDV RNA in postmortem brain samples from individuals with mental disorders. This study reported the detection of BDV P gene transcripts in 9 out of 17 patients with schizophrenia and 2 out of 5 patients with bipolar disorder.

Can BDV Contribute to the Pathophysiology of Human Mental Disorders?

The data discussed above provide strong evidence that BDV can infect humans and persist in the CNS. The establishment of an association between BDV and certain neuropsychiatric disorders awaits confirmation from more comprehensive molecular epidemiological studies. However, it should be emphasized that finding such an association does not prove the contribution of BDV to the pathophysiology of these mental disorders. In addition, BDV has been associated with both schizophrenia and affective disorders, raising the question of how the same infectious agent could be involved in two different mental disorders.

Schizophrenia and affective disorders are chronic and complex CNS diseases, manifested by multiple signs and symptoms that tend to recur, often in a cyclical fashion [3,164]. Epidemiological data indicate that environmental factors, such as stress, contribute to these disorders [3,41,71,72,164,178]. There is compelling evidence that viral infection of the brain represents an important risk factor [227]. Moreover, findings in BDV-infected animals suggest that the biology of BDV is compatible with its potential role in these mental disorders [198]. BDV-infected animals display a diverse range of symptoms, including neurobehavioral alterations with cyclic episodes. The remarkable heterogeneity in disease phenotype associated with BDV infection depends on both host and viral factors, as well as other exogenous factors. Thus, stress can contribute to recurrent disease episodes in BDV chronically infected animals [83,134].

Neurostructural abnormalities have been associated with schizophrenia and affective disorders. These affect several brain regions but are predominantly localized to medial temporal lobe structures. However, functional alterations suggest that the disease process may also affect other brain areas, involve integrative sensory function and motor coordination [227]. Abnormalities within the hippocampal formation are frequently observed in these disorders [3,18,191]. Studies using high resolution volumetric magnetic resonance imaging have shown that affective disorders are associated with structural changes in the hippocampus [191]. It has been suggested that the long-term overexpression of glucocorticoids, frequently seen in patients with major depression, could cause degeneration of hippocampal neurons, which express high levels of glucocorticoids receptors [178]. In the case of schizophrenia, hippocampal alterations are thought to reflect an altered process of cell migration during brain development, possibly triggered by an early viral infection [18]. BDV has a predominant tropism for limbic system structures [37,78,140] and perinatal infection causes hippocampal damage. Interestingly, magnetic resonance imaging studies have suggested that cerebral abnormalities occur more frequently in BDV-seropositive psychiatric patients [11,218]. Moreover, PTI-NB rats display cerebellar hypoplasia. The cerebellum is linked with limbic structures through multisyn-

aptic circuits, which have been implicated in mood regulation [196]. There is also evidence of a decreased size of the cerebellum in patients with affective disorders [63,152,220].

Altered neurotransmitter functions affecting mainly catecholamines and especially dopaminergic systems have been implicated in the pathophysiology of human mental diseases [80,164,226]. This altered neurotransmission activity is thought to affect the regulation of limbic system functions, which, in turn, can lead to the various endocrine disturbances observed in these disorders [71,72]. BDV infection causes distinct CNS dopamine disturbances [199,200]. BDV can also affect other neurotransmitter systems [122], or the expression of neurotrophic factors required to maintain brain homeostasis. It is worth noting that BDV infection induces a prominent and chronic astrocytosis that was also observed in the autopsy brain samples from human cases identified as positive for BDV antigen and RNA [51]. BDV-induced disturbances in astrocyte functions may contribute to neuronal dysfunction by affecting complex interactions within neural networks (see section 3.4).

Major human mental disorders, including schizophrenia and affective disorders, likely involve a concert of genes with varying penetrance among individuals that interact with exogenous factors to generate a variety of clinical phenotypes [164]. BDV can represent an exogenous factor contributing to these disorders.

Viral variants, even single amino acid substitutions, can exhibit remarkable differences in their expression pattern in cells and tissues of the infected host, that are associated with diverse disease phenotypes [98,210]. Similarly, genetic differences among individuals play a critical role in the outcome of a viral infection [32]. Thus, it is conceivable that depending on the individual's genetic vulnerability and the properties of specific viral variants, BDV infection could contribute to distinct types of mental disorders.

The source of BDV and routes for human infection are also germane to the role of BDV in neuropsychiatric disorders. Evidence suggests that the nose is a main site of virus entry in animals [172]. Sporadic evidence has suggested the possibility of transmission from BDV-infected animals to humans [14,222]. The close genetic relationship between BDV sequences derived from humans and animal strains provides support for this hypothesis. In this regard, it is of interest that domestic cats are susceptible to BDV, representing potential carriers of BDV [129,145]. The detection of BDV RNA in a significant percentage (4.2 to 5%) of blood donors' PBMC raises the concern of a possible transmission by blood transfusion [107]. As with other neurotropic viruses, a small number of BDV-infected cells present in a blood sample could reach the brain and establish a persistent infection within the CNS. Depending on the genetic susceptibility of the host and on viral factors, this persistent infection may have diverse clinical consequences, manifested after a variable period of incubation. One should keep in mind that stress, a common finding in psychiatric patients, can induce immunosuppression by altering glucocorticoids levels [144]. This, in turn, could enhance susceptibility to infectious agents, including BDV.

The molecular analysis of human biological samples to detect BDV poses considerable technical difficulties. Comprehensive molecular epidemiological studies aimed at determining the prevalence of BDV in neuropsychiatric patients from different geographic areas will help to evaluate the significance of BDV as a potential human pathogen. These studies will require the use of standardized methods for the detection of viral antibodies and RNA sequences in human biological samples, as well as uniform criteria for the clinical diagnoses.

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