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Bornavirus immunopathogenesis in rodents: models for human neurological diseases

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Although the question of human BDV infection remains to be resolved, burgeoning interest in this unique pathogen has provided tools for exploring the pharmacology and neurochemistry of neuropsychiatric disorders potentially linked to BDV infection. Two animal models have been established based on BDV infection of adult or neonatal Lewis rats. Analyis of these models is already yielding insights into mechanisms by which neurotropic agents and/or immune factors may impact developing or mature CNS circuitry to effect complex disturbances in movement and behavior.

Keywords: Borna disease virus; neurotropism; humoral and cellular immune response; Th_1-Th_2 shift; apoptosis; dopamine; cytokines

Introduction

Borna disease virus (BDV), the prototype of a new family, *Bornaviridae*, within the nonsegmented negative-strand RNA viruses, infects the central nervous system (CNS) of warmblooded animals to cause behavioral disturbances reminiscent of autism, schizophrenia, and mood disorders (Lipkin *et al*, 1995). BDV is not lytic *in vitro* or *in vivo*, replicates at lower levels than most known viruses and is dissimilar in nucleic acid and protein sequence to other infectious agents (de la Torre, 1994; Schneemann *et al*, 1995). The molecular biology of BDV is unusual in many respects including a nuclear localization for replication and transcription, overlap of open reading frames and transcription units, posttranscriptional modification of subgenomic RNAs, and marked conservation of coding sequence across a wide variety of animal species and tissue culture systems. Natural infection has been confirmed in horses, sheep, cattle, dogs, birds and cats. Primates can be infected experimentally (Stitz *et al*, 1980). This wide host range suggests that humans are likely to be susceptible to BDV infection; however, there is no consensus concerning the role of BDV in human disease. Although some investigators report an increased prevalence of BDV infection in mood disorders and schizophrenia (Amsterdam *et al*, 1985; Bode *et al*, 1988, 1992, 1993; Fu *et al*, 1993; Kishi *et al*, 1995; Waltrip II *et al*, 1995), others have not succeeded in replicating these findings (Iwata *et al*, 1998; Kubo *et al*, 1997; Lieb *et al*, 1997; Richt *et al*, 1997). Here we review two rodent models of BDV infection that provide insight into mechanisms by which neurotropic viruses may impact CNS development and function to effect complex disturbances in behavior.

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Neurotropism and persistence

Neurotropism of BDV is likely to be multifactorial. The integrity of the humoral immune response is critical to restriction of virus to neural compartments (Stitz *et al*, 1998); however, replication is still higher in limbic structures in animals with compromised humoral immunity; thus, additional factors must contribute to neurotropism. One means by which preferential replication of BDV in limbic structures might occur is through restricted distribution of the enzymatic machinery required for its lifecycle. The phosphoprotein of BDV (P) is predicted by analogy to phosphoproteins of other nonsegmented negative-strand RNA viruses to serve as a transcriptional activator (de la Torre, 1994; Schneemann *et al*, 1995). It also contains potent nuclear localization signals (Shoya *et al*, 1998;

Schwemmle *et al*, 1999) and interacts with two other BDV proteins, the nucleoprotein and X-protein to affect their intracellular distributions (Schwemmle *et al*, 1998). Thus, P may also have additional functions related to nucleocasid assembly and/or protein trafficking within the cell. BDV P is phosphorylated primarily by the epsilon isotype of protein kinase C (PKCε) (Schwemmle *et al*, 1997). Interestingly, the regional distributions of PKCε (Saito *et al*, 1993) and BDV in rat brain (Narayan *et al*, 1983a) are similar, suggesting the possibility that the localization of PKCε, through its phosphorylation effects, may influence the tropism of BDV for limbic circuitry.

In many CNS viral infections, the presence of an intact immune response results in either viral clearance or host mortality. This is not the case in BDV, where infection may persist in the presence of a transient but robust immune response. Persistence may be due to induction of Th_1 tolerance. Whereas lymphocytes isolated from brains of acutely infected rats have potent cytolytic activity, lymphocytes from brains of chronically infected rats do not lyse BDV-infected target cells (Sobbe *et al*, 1997). Induction of BDV-specific tolerance in chronic infection may reflect the timecourse for presentation of viral antigens in the thymus (Rubin *et al*, 1995). Alternatively, Th_1 cells may become anergic or undergo apoptosis due to presentation of BDV antigens in brain without essential costimulatory signals (Karpas *et al*, 1994; Khoury *et al*, 1995; Schwartz, 1992). Support for the latter hypothesis is found in the observation that apoptosis of perivascular inflammatory cells is most apparent at 5–6 weeks post infection (p.i.), coincident with the onset of decline in encephalitis (Hatalski *et al*, 1998a).

In an effort to explore differences in host gene expression during persistent infection that might be linked to tolerance, subtractive cloning methods were applied to analysis of RNA from brains of acute and persistent adult infected rats. The persistently infected rats had a dramatic increase in mRNAs encoding immunoglobulin – a finding that suggested that Th_1 tolerance might reflect a Th_1–Th_2 shift in the immune response. This hypothesis was confirmed by RNase protection assays that demonstrated a shift in the brain cytokine mRNA profile from Th_1–Th_2 cytokines and an isotype switch in peripheral blood from IgG to IgE (Hatalski *et al*, 1998a,b). Although the enhanced humoral immune response in chronic disease does not promote viral clearance, it may play an important role in limiting viral gene expression (Hatalski *et al*, 1998a). Complementary work by Hatalski *et al* (1998b) in the chronic phase of BD reported increases in intra-CNS production of IgG antibodies that parallel increases of antibodies with neutralizing activity against BDV in peripheral blood. Definitive evidence that humoral immunity contributes to BDV tropism emerged from recent work by Stitz *et al* where passive transfer of neutralizing antibodies resulted in limitation of viral replication within the CNS (Stitz *et al*, 1998). Similar results have been reported in other viral systems; for example, passive transfer of virus-specific antibodies limits viral replication in the CNS following infection with murine hepatitis virus type-4 (Buchmeier *et al*, 1984) or measles virus (Liebert *et al*, 1990), and induces clearance of virus following infection with rabies (Dietzschold *et al*, 1992) or Sindbis virus (Levine *et al*, 1991).

Adult Lewis rat infection

Bornavirus neuropathogenesis has been studied primarily in adult immunocompetent rodents and ungulates where infection results in dramatic disturbances in behavior, limbic circuitry, and monoamine neurotransmitter systems. These models are intriguing; however, they are associated with marked CNS inflammation, loss of brain mass and gliosis and may be less relevant to neuropsychiatric diseases than those in neonatally infected rats where BDV induces subtle disturbances of behavior and dysgenesis of the cerebellum and hippocampus without robust inflammatory cell infiltration.

As in autism (Anderson, 1994; Ernst *et al*, 1997), schizophrenia (Cooper *et al*, 1991), and mood disorders (Hamner and Diamond, 1996; Kelsoe *et al*, 1996; Partonen, 1996), disorders of movement and behavior in adult BD rats are linked to distinct changes in CNS dopamine systems (Solbrig *et al*, 1994, 1995, 1996a,b, 1998) and may be further linked to serotonin abnormalities (Solbrig *et al*, 1995). The immune-mediated disorder in adult infected rats presents clinically as hyperactivity and exaggerated startle responses 10–14 days after intracerebral infection (Narayan *et al*, 1983a). The acute phase coincides with infiltration of monocytes into the brain, particularly in areas of high viral burden including the hippocampus, amygdala and other limbic structures (Carbone *et al*, 1987). Two to three weeks later, rats show high grade stereotyped motor behaviors (the continuous repetition of behavioral elements such as sniffing, chewing, scratching, grooming, and self-biting), dyskinesias, dystonias, and flexed seated postures (Solbrig *et al*, 1994), in parallel with the widespread distribution of virus in limbic and prefrontal circuits. Five to ten per cent of animals become obese, achieving body weights up to 300% of normal (Ludwig *et al*, 1988).

Central dopamine systems of adult-infected BD animals are more sensitive to dopamine agonists and antagonists than normal rats. Infected animals have increased locomotor and stereotypic behavior following administration of the mixed-acting dopamine agonist, dextroamphetamine (Solbrig *et al*, 1994). Similarly, enhanced locomotion and stereo-

typies are seen in response to the dopaminergic reuptake inhibitory effects of cocaine, indicating dose-dependent potentiation of dopamine neurotransmission (Solbrig *et al*, 1998). The movement and behavior disorder is improved following treatment with selective dopamine antagonists; whereas D2-selective antagonists (e.g., raclopride) do not affect locomotor responses in BD rats, high doses of selective D1 antagonists (e.g., SCH23390) and atypical dopamine blocking agents with mixed D1 and D2 antagonist activity such as clozapine selectively reduce locomotor activity in BD rats but not in controls (Solbrig *et al*, 1994).

HPLC analyses reveal abnormal neurochemistry in adult-infected BD rats. Levels of dopamine and its major metabolite, dihydroxyphenylacetic acid (DOPAC), are reduced in structures that receive dopaminergic terminals including striatum, nucleus accumbens, and olfactory tubercle (Solbrig *et al*, 1994). Interestingly, although levels of dopamine and DOPAC are also reduced in prefrontal cortex, the ratio of DOPAC to dopamine is increased suggesting enhanced turnover of dopamine in this structure (Solbrig *et al*, 1996a). Tyrosine hydroxylase-immunoreactive cells are depleted in substantia nigra and ventral tegmental area (tyrosine hydroxylase is the rate limiting enzyme in dopamine synthesis and may be used to identify cells that produce dopamine) (Solbrig *et al*, 1994). Taken together, these results suggest partial dopaminergic deafferentation with compensatory metabolic hyperactivity in nigrostriatal and mesolimbic dopamine systems. At the receptor level, both pre- and postsynaptic sites of the dopamine transmitter system appear to be damaged in striatum (caudate-putamen and nucleus accumbens). Dopamine uptake sites, as measured by binding of mazindol, are reduced in nucleus accumbens (Solbrig *et al*, 1996b) and caudate-putamen (Solbrig *et al*, 1998). D2 (but not D1) receptor binding is markedly reduced in caudate-putamen; D2 and D3 receptor binding are reduced in nucleus accumbens (Solbrig *et al*, 1994, 1996a,b). In contrast, postsynaptic dopamine receptors (D1, D2, D3) remain intact in prefrontal cortex (Solbrig *et al*, 1996a). The basis for this targeted pathogenesis remains unclear; however, within caudate-putamen and nucleus accumbens the postsynaptic dopamine lesions appear to be confined to receptors expressed from spliced messages (D2, D3) rather than unspliced messages (D1). Given that BDV requires the host cell splicing machinery for expression of its genome it has been proposed that competition for splicing machinery may contribute to selective pathology within dopamine circuits (Solbrig *et al*, 1994).

Although the increased locomotor activity, stereotypic behaviors and dyskinesias of the adult BD model are linked to distinct disturbances in dopaminergic pathways, additional neuromodulator abnormalities have been found. Levels of mRNA for somatostatin, cholecystokinin, and glutamic acid decarboxylase are reduced during the acute phase and recover toward normal in the chronic phase of disease (Lipkin *et al*, 1988). The cholinergic system, a major participant in sensorimotor processing, learning, and memory, also appears to be affected in adult infection. A decrease in the number of choline acetyltransferase-positive fibers has been observed to begin as early as day 6 p.i. and progress to nearly complete loss of cholinergic fibers in hippocampus and neocortex by day 15 p.i. (Gies *et al*, 1998). Preliminary work on dysregulation of serotonin and norepinephrine systems suggests metabolic hyperactivity of serotonin (as evidenced by modest increase in the metabolite 5-hydroxyindoleacetic acid [5HIAA]) in striatum and of norepinephrine (as evidenced by a small increase in 3-methoxy-4-hydroxyphenethyleneglycol [MHPG]) in prefrontal and anterior cingulate cortex regions (Solbrig *et al*, 1995). These changes may reflect compensatory upregulation or heterotypic sprouting following partial loss of dopaminergic afferents to these brain regions. Selective effects of BDV on serotonin and norepinephrine pre- or postsynaptic receptors have not yet been investigated. Pharmacological and neurotransmitter-specific molecular probes have also been used to characterize endogenous opioid systems in the adult rat model. Infected animals respond abnormally to the opiate antagonist, naloxone, with hyperkinesis and seizures, and also demonstrate increases in striatal preproenkephalin mRNA at 14 and 21 days (Fu *et al*, 1993b), and 45 days after BDV infection (Solbrig and Lipkin, personal communication). However, the mechanisms by which these changes in endogenous opioid systems occur are unclear. The marked CNS inflammation in adult-infected rats makes it difficult to determine whether monoamine, cholinergic, and opiatergic dysfunction in BD results from direct effects of the virus, virus effects on resident cells of the CNS, or a cellular immune response to viral gene products.

It is difficult to establish a direct parallel between the disturbances observed in adult infected rats and a single human CNS disorder because infected rats have an evolving syndrome. Early phases of disease are reminiscent of the hyperactivity observed in bipolar disorder or attention deficit disorder. Such interpretations are interesting historically for their influence on investigators pursuing connections between BDV and human diseases; however, the most compelling aspects of the syndrome from the vantage point of establishing models for human disease instead focus on movement disorders. These simple and complex behaviors clinically and pharmacologically resemble tardive dyskinesia, a common iatrogenic condition that follows use of psychotropic medications, the 'on-off' phenomen-

on found in late stages of Parkinsonism, and are consistent with some descriptions of postencephalitic Parkinsonism (Solbrig *et al*, 1999).

Neonatal Lewis rat infection

Neonatal rat infection may provide an even more intriguing model for neuropsychiatric disorders than adult rat infection. Indeed, the cerebellar and hippocampal dysgenesis that is observed in neonatally-infected animals (Carbone *et al*, 1991; Narayan *et al*, 1983a) is consistent with the more subtle neurodevelopmental abnormalities reported by some investigators in autism (Kemper and Bauman, 1993), schizophrenia (Altshuler *et al*, 1987; Fish *et al*, 1992), and affective disorders (Soares and Mann, 1997). Neonatally infected animals display a wide range of physiologic and neurobehavioral disturbances. A study of behavioral and cognitive changes in neonatally infected Wistar rats found spatial and aversive learning deficits, increased motor activity, and decreased anxiety responses (Dittrich *et al*, 1989). Similar deficits in spatial learning and memory were reported in neonatally infected Lewis rats 23–73 days p.i. (Carbone *et al*, 1996). More recently, play behavior has been reported to be abnormal in the neonatal model, with decreases in both initiation of nondominance-related play interactions and in response to initiation of play by noninfected, age-matched control animals or by infected littermates (Pletnikov *et al*, 1999). Neonatally infected animals are smaller than uninfected littermates (Carbone *et al*, 1991; Bautista *et al*, 1994). The basis for runting is unclear as levels of glucose, growth hormone, and insulin-like growth factor-1 (Bautista *et al*, 1994) are normal. The amount of food ingested (Bautista *et al*, 1995) is similar in uninfected and infected rats although the latter have a heightened taste preference for salt solutions (Bautista *et al*, 1994).

CNS dysfunction in neonatally infected animals has been proposed to be linked to direct viral effects on morphogenesis of the hippocampus and cerebellum, two structures in rodents that continue to develop after birth. Carbone and colleagues found a quantitative relationship of limbic pathology to behavioral abnormalities in the neonatal infection model; the extent of neuronal loss in dentate gyrus appeared to be correlated with the severity of spatial learning and memory deficiencies in neonatally-infected Lewis rats (Carbone *et al*, 1996). Although overt ambulatory or cerebellar dysfunction has not been reported (Carbone *et al*, 1991), we found impairments in balance and coordination during a sensitive dowel-walking task (Hatalski, 1996). Because the cerebellum undergoes substantial postnatal development in many mammals, it is particularly vulnerable to injury from perinatal virus infection (Monjan *et al*, 1971, 1973; Oster-Granite and Herdon, 1985). Confirmation of subtle abnormalities in motor coordination in neonatally-infected rats would provide a functional correlate to anatomic alterations in cerebellum. However, further studies are needed to evaluate the mechanisms by which early postnatal exposure to BDV induces functional damage in either cerebellar or limbic circuitry.

Early reports indicated that there was no cellular inflammatory response following neonatal infection (Carbone *et al*, 1991; Stitz *et al*, 1995; Gosztonyi and Ludwig, 1995), a phenomenon ascribed to the immaturity of the rat immune system in the postnatal period. Humoral immune response to BDV in neonatally-infected animals has also been reported to be restricted, with anti-BDV antibody titers remaining below 1 : 10 through 133 days p.i. (Carbone *et al*, 1991). However, marked astrocytosis has been noted (Carbone *et al*, 1991; Gonzalez-Dunia *et al*, 1996; Bautista *et al*, 1995) in dentate gyrus and cerebellum, suggesting alternate, noninflammatory pathways for glial activation. Higher levels of message for tissue factor (TF) are found in infected hippocampus. TF is a member of the class II cytokine receptor family primarily produced by astrocytes that plays important roles in cellular signal transduction, brain function, and neural development through its effects on coagulation protease cascades. Although this may be one mechanism by which BDV may alter CNS development (Gonzalez-Dunia *et al*, 1996), cerebellar changes cannot be explained by this mechanism, as TF upregulation is not observed in cerebellum despite prominent astrocytosis. Furthermore, BDV infection of astrocytes appears to be required for TF upregulation (Gonzalez-Dunia *et al*, 1996), and cerebellar astrocytes are reported to be spared from BDV infection, at least through 30 days following neonatal infection (Bautista *et al*, 1995).

Persistent tolerant BDV infection of neonatal rats is linked to hippocampal and cerebellar disorganization (Narayan *et al*, 1983b; Stitz *et al*, 1995); however, cytoarchitectonic anomalies in other limbic regions have not been extensively explored. Dentate gyrus involution is evident along with the appearance of reactive glial cells (Carbone *et al*, 1991), suggesting more direct pathways of viral cytopathic injury. Cerebellar size is reduced, and there is evidence of reactive astrocytosis as demonstrated by glial fibrillary acidic protein (GFAP) reactivity as early as 3 days p.i., preceding the identification of BDV proteins in the cerebellum. Furthermore, reactivity of cerebellar astrocytes and loss of cerebellar granule cells occurs without signs of infection in those cell populations at all timepoints through to 30 days p.i. Curiously, Purkinje cells appeared to be the predominant cerebellar cell population demonstrating BDV proteins, although these cells did not appear to be selectively lost through day 30 p.i. (Bautista *et al*, 1995). The

mechanism by which astrocytes are activated in the absence of infection, be it directly by BDV or indirectly through elaboration of soluble factors by other cell types, is not known. Nonetheless, given the role of astrocytes in guiding migration of granule cells during cerebellar development, an assessment of the frequency of astrocyte reactivity in conjunction with studies of apoptosis in limbic structures may elucidate the relative contributions of migrational failure and programmed cell death in pathogenesis of neonatal infection.

Although previous work suggests subtle functional disturbances of limbic circuitry based upon analysis of complex learning behaviors, memory capacities, and emotional responses, the evolution of such disturbances and the mechanisms by which BDV induces their underlying neuropathology without invoking infiltrating inflammatory elements remains to be determined. In an effort to more fully define the nature and unfolding of the neurologic syndrome in neonatally-infected Lewis rats, and to understand the mechanisms of neuropathogenesis in the neonatal model, we established neonatal infection in Lewis rats and serially assessed shifts in neuroanatomy, neurobehavior, and regional gene expression (Hornig *et al*, 1999).

Locomotor activity and stereotypes were assessed in neonatally infected animals 4, 6 and 12 weeks p.i. Results of locomotor activity analyses indicated an overall significant increase in neonatally infected groups relative to controls at all timepoints tested. Analysis of data extrapolated from previous studies of locomotor activity in adult infected animals (Solbrig *et al*, 1994, 1996b, 1998) revealed that the degree of heightened exploratory locomotor activity found at baseline in neonatally infected animals at 6 and 12 weeks postinfection is greater than in adult infected animals. Locomotor activity across the 90 min observation period differed for all neurally infected groups relative to noninfected controls. At 4 weeks following neonatal infection, animals had prolonged behavioral inhibition upon introduction to the novel environment (first 30 min interval). These findings are consistent with greater anxiety in novel situations and suggests dysfunction of the amygdala. At the 60 and 90 min intervals, infected animals had greater mean activity measures than controls. Additionally, infected animals showed no attenuation in exploratory activity at 60 and 90 min, consistent with spatial memory deficits and hippocampal dysfunction. Stereotypic behaviors were also increased in neonatally infected groups relative to noninfected controls.

Serial analyses of differential gene expression of cytokines, neurotrophic factors, and apoptosis-related proteins were pursued by RNase protection assays to assess potential contribution of soluble factors to neuropathogenesis. Possible mechanisms of cytokine-mediated damage in the context of the developing brain include: direct effects on neuronal elements; activation or suppression of second messenger/intracellular signaling pathways; induction of shifts in excitotoxic elements such as quinolinic acid or acute phase proteins such as neopterin or β-2-microglobulin; direct alterations of neuronal function (e.g., inhibition of long-term potentiation in hippocampus); activation or suppression of glial cells; or alteration of glial cell proliferation or differentiation (including expression of adhesion molecules such as the integrins) (Benveniste, 1997; Mehler *et al*, 1996). Given that the postnatal expression of neuronotrophic cytokine and cytokine receptor mRNAs in brain differs for each cytokine (Benveniste, 1997), and that the sensitivity of neuronal populations to the trophic or apoptosis-inducing effects of cytokines changes during development, wide variation in the patterns of virus-induced, cytokine-related damage would be expected, depending on the relative maturity of the evolving nervous system at the time of infection. In addition, cell loss induced by either BDV or developmentally-programmed changes may alter the capacity of resident CNS cells to both produce and respond to neuronotrophic cytokines. One means by which a virus might disrupt neural function and development in the absence of inflammation is through the induction of neuronotrophic cytokines. Neuronotrophic cytokines comprise a burgeoning set of immunoregulatory molecules, including the hematolymphoietic factors (e.g., interleukins, tumor necrosis factor family, interferons), the TGF-β superfamily factors (including TGF-β1, 2, 3; GDNF), and the classic neurotrophic factors (NGF, BDNF, NT3, NT4/5). A large subset of the neuronotrophic, hematolymphoietic cytokines may be categorized according to their origin from one of two types of T-helper cells: Th_1 (cell-mediated immunity and stimulation of antigen-presenting cells) or Th_2 (humoral or B-cell mediated immunity).

Brains of infected and noninfected animals were removed at 2, 4, 6, 12, and 24 weeks p.i. and dissected to collect hippocampus, amygdala, cerebellum, prefrontal cortex, and nucleus accumbens. RNA samples from individual brain regions were subjected to RNase protection assay to quantitate level of transcripts encoding cytokines interleukin (IL)-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, TNF-α, TNF-β, IFN-γ, and TGF-β and housekeeping genes L32 and GAPDH. Infected rats had higher levels of mRNAs for cytokine products of CNS macrophages/microglia (IL-1α, IL-1β, IL-6, TNF-α) in hippocampus, amygdala, cerebellum, prefrontal cortex, and nucleus accumbens. Elevated levels of these proinflammatory cytokines were first apparent at 2 weeks, peaked at 4 weeks, then declined at 6 and 12 weeks. Alterations in other proinflammatory cytokines, including IL-2, IL-3, TNF-β, and IFN-γ, were not observed. The fact that cell populations other than macrophages or microglia – T cells, B

cells, mast cells, bone marrow stromal cells – are the primary sources for the proinflammatory cytokines that remained static following neonatal infection suggests a selective effect of BDV on cells of microglial or macrophage lineage.

Shifts were also observed in gene expression of neurotrophic factors; however, in contrast to the diffuse alterations found in gene expression of cytokine mRNAs, shifts in neurotrophic factor mRNAs were restricted to hippocampus. Decreased mRNAs for BDNF and NT3 were prominent in hippocampus by 4 weeks p.i., but were still evident by 12 weeks p.i. Although decreased NT3 mRNA may reflect loss of the granule cell population in dentate gyrus, the role of BDNF in maintaining viability of cells suggests that its downregulation may be a more essential step in neonatal BDV pathogenesis.

Apoptosis, or programmed cell death, is a mechanism in which cells undergo chromosome condensation, DNA degradation, and morphologic change in the nuclear membrane (Wyllie, 1995). Apoptosis plays an important role in CNS development and response to neuronal injury (Bredesen, 1995). It is conceivable that abnormal regulation of apoptosis, either failure of normal apoptotic sequences to proceed or excessive activity, may contribute to abnormal CNS architecture in neonatal infections with BDV or other neurotropic viruses. Furthermore, apoptosis of antigen-specific lymphocytes might provide an explanation for the immunotolerant state following neonatal BDV infections. Anergy or apoptosis of T cells may result if their stimulation by antigen presenting cells resident to the CNS occurs in the absence of costimulatory signals required for immune activation, such as MHC Class II antigens (Munn *et al*, 1996). Various stimuli, such as binding of TNF-α to its receptor, can trigger apoptosis; proteins such as the bcl-2 and bax proteins, NF-kappaB and ICE-related proteases have also been shown to play important roles in regulating apoptosis. TNF-α also stimulates apoptosis in a wide variety of cell types (Benveniste, 1997). Furthermore, a host of excitants or neurotoxins including arachidonic acid, platelet-activating factor, free radicals (NO, O_2^-), glutamate, quinolinate, cysteine, cytokines (TNF-α, IL1-β, IL-6), amines, and as yet unidentified factors arising from stimulated macrophages and possibly reactive astrocytes may influence apoptosis by excessive activation of N-methyl-D-aspartate (NMDA) receptors (Lipton, 1996). Interestingly, Gosztonyi and Ludwig have proposed that the targeted pathology of BDV for two hippocampal cell layers, stratum oriens and stratum radiatum, may be due to their rich concentration of glutamate and aspartate receptors (Gosztonyi and Ludwig, 1995). Our RNase protection assay analyses revealed complex alterations in brain of mRNAs encoding factors associated with apoptosis. Levels of mRNA for FAS and ICE (caspase-1), two promoters of apoptosis, were increased. Levels of mRNA for bcl-x, a factor that inhibits apoptosis, were decreased. Maximal shifts were observed at 4 and 6 weeks p.i., closely paralleling the increases in proinflammatory cytokines noted earlier.

Anatomic studies were consistent with previous reports (Carbone *et al*, 1991; Narayan *et al*, 1983b) in demonstrating loss of dentate gyrus granule cells and disorganization of cerebellar granule cell layer. However, new findings included inflammatory cellular infiltrates at week 4 and nearly complete loss of cerebellar Purkinje cells by week 6. Interestingly, the inflammatory infiltrates were restricted to the motor, parietal and temporal cortex; only rare inflammatory cells were detected in dentate gyrus or cerebellum, the regions where architecture was most disturbed. Terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling (TUNEL) was observed in cerebral cortex and dentate gyrus peaking at 4 weeks p.i. and in granule cell layer of cerebellum of neonatally infected rats at weeks 4 and 6 p.i. Although apoptosis is described in normally developing rat hippocampus as late as day 7 to 10 of postnatal life, it is not found at later timepoints (Toth *et al*, 1998). The anatomic location of the apoptotic cells and the absence of inflammatory cells in hippocampus and cerebellum suggests that at least some aspects of neuropathology in neonatal infection reflect apoptosis rather than cell-mediated, specific immunity to BDV. Efforts are underway to determine which cells in hippocampus and cerebellum express soluble mediators that might promote apoptosis. Microglial cells are candidates for this activity. Recent studies indicate that they are activated in hippocampus and cerebellum of neonatally infected rats (Hornig *et al*, 1999).

Summary

The issue of human BDV infection remains controversial. Nonetheless, recent focus on this intriguing agent has led to establishment of small animal models for studies in BDV pathogenesis that may provide insights into human neuropsychiatric disorders. The two systems presented here, adult and neonatal Lewis rat infection, illustrate the complexities of analyzing neural:immune interactions in a developmental context. Together, they provide powerful tools for exploring the effects of viral and immune factors on CNS development and dynamic models for parsing the anatomy, pharmacology and neurochemistry of specific neuropsychiatric syndromes.

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