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Review

The pathogenesis of bornaviral diseases in mammals

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Abstract

Natural bornavirus infections and their resulting diseases are largely restricted to horses and sheep in Central Europe. The disease also occurs naturally in cats, and can be induced experimentally in laboratory rodents and numerous other mammals. Borna disease virus-1 (BoDV-1), the cause of most cases of mammalian Borna disease, is a negative-stranded RNA virus that replicates within the nucleus of target cells. It causes severe, often lethal, encephalitis in susceptible species. Recent events, especially the discovery of numerous new species of bornaviruses in birds and a report of an acute, lethal bornaviral encephalitis in humans, apparently acquired from squirrels, have revived interest in this remarkable family of viruses. The clinical manifestations of the bornaviral diseases are highly variable. Thus, in addition to acute lethal encephalitis, they can cause persistent neurologic disease associated with diverse behavioral changes. They also cause a severe retinitis resulting in blindness. In this review, we discuss both the pathological lesions observed in mammalian bornaviral disease and the complex pathogenesis of the neurologic disease. Thus infected neurons may be destroyed by T-cell-mediated cytotoxicity. They may die as a result of excessive inflammatory cytokine release from microglia. They may also die as a result of a 'glutaminergic storm' due to a failure of infected astrocytes to regulate brain glutamate levels.

Keywords: Bornavirus, encephalitis, microglial activation, astrocytes, glutamate, excitotoxicity, mammals.

Introduction

Bornaviruses have long been known to cause meningoencephalitis in horses and sheep in parts of Central Europe (Metzler *et al.*, 1976; Durrwald and Ludwig, 1997; Richt *et al.*, 2000). Subsequent studies have demonstrated that they induce a similar disease in experimentally infected laboratory rats and mice (Narayan *et al.*, 1983a, b; Kao *et al.*, 1984). The course of the rodent disease is however dependent upon age, such that adult rats develop an acute encephalitis while newborns develop multiple neurodevelopmental disorders. Other bornavirus-infected rodents such as gerbils fail to show these age-related differences. Additionally, a bornavirus from variegated squirrels (Variegated squirrel bornavirus, VSBV-1) can cause an acute lethal encephalitis in humans (Hoffmann *et al.*, 2015). More significantly, it was believed for many years that bornaviruses contributed to human mental illness (Bode and Ludwig, 2003). This concept has been largely discredited. In contrast, avian bornaviral infection of parrots and waterbirds results in proventricular dilatation disease as well as encephalitis (Honkavuori *et al.*, 2008; Kistler *et al.*, 2008; Rubbenstroth *et al.*, 2014). An uncharacterized bornavirus also appears to cause a naturally occurring acute paralytic syndrome in ostriches (Malkinson *et al.*, 1993; Ashash *et al.*, 1996). The goal of this review is to provide an overview of the pathogenesis of bornavirus-mediated neurologic disease in mammals in light of these recent findings.

Bornaviruses

Bornaviruses (order *Mononegavirales* family *Bornaviridae*,) are enveloped non-segmented, single stranded, negative sense

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RNA viruses whose prototype member is Borna disease virus 1 (BoDV-1) (Lipkin *et al.*, 2011). BoDV-1 primarily infects mammals but has been detected in birds (Malkinson *et al.*, 1993; Berg *et al.*, 2001). Bornaviruses have a unique genome organization among the *Mononegavirales*. The 8.9 kb genome contains six open reading frames that encode six viral proteins, N, P, M, G and L plus a small X protein that overlaps the P reading frame. To maximize the use of the genome, BoDV-1 employs the RNA splicing machinery for gene expression (Cubitt *et al.*, 1994; Schneider *et al.*, 1994). Three transcription start sites and four termination sites have been identified as well as the use of splicing to generate additional mRNAs (Schneemann *et al.*, 1994; Ludwig, 2008). N, P and L proteins together with the viral RNA form a ribonucleoprotein (RNP) complex (Fig. 1). Once within the cytoplasm, the RNP complex translocates into the nucleus (Jamali *et al.*, 2011; Honda and Tomonaga, 2013). Viral replication and transcription occur within the nucleus. Only a small number of infectious particles are released from bornavirus-infected cells (Gonzalez-Dunia *et al.*, 1998; Tomonaga *et al.*, 2002).

For many years, BoDV-1 was the only known member of the *Bornaviridae*. It shows little genetic variation with 4.1% diversity in nucleotides and 1.5–3% diversity in the amino acid sequences of its N- and P-proteins (Formella *et al.*, 2000; Lipkin *et al.*, 2011). The finding of multiple bornavirus species in parrots in 2008 changed that situation. Thus the known *Bornaviridae* have expanded from a single conserved mammalian virus to a highly diverse family of at least six viral species (Kuhn *et al.*, 2015). *Mammalian 1 bornavirus* encompasses classical Borna disease virus- BoDV-1 and BoDV-2. Recently, VSBV-1 has been identified and may be a new species. *Parrot 1 bornavirus* encompasses psittacine bornaviruses 1, 2, 3, 4 and 7; *Parrot 2 bornavirus*, encompasses psittacine bornavirus 5; *Passeriform 1 bornavirus* (canary bornaviruses); *Passeriform 2 bornavirus* (estrilidid finch bornavirus); *waterbird 1 bornavirus* (aquatic bird bornaviruses -1 and -2) have also been described. At least two bornaviruses have also been identified in snakes (*Elapid 1 bornavirus* and an unclassified virus) and multiple endogenous bornaviral sequences have been identified in mammals including people (Horie *et al.*, 2010, 2013).

Borna disease in mammals

Natural Borna disease affects horses and sheep in central Europe. It occasionally affects other domestic mammals such as donkeys, goats and cattle. It has been recorded in rabbits, some zoo animals and dogs (Staeheli *et al.*, 2000). Cases of 'staggering disease' in cats have also been attributed to infection by BoDV-1 (Wensman *et al.*, 2014). It is believed that these naturally occurring cases result from infection acquired from the urine of shrews.

The bicolored white-toothed shrew (*Crocidura leucodon*) is a reservoir host species for BoDV-1 within endemic regions of central Europe and develops an asymptomatic infection (Sprankel *et al.*, 1978; Puorger *et al.*, 2010; Nobach *et al.*, 2015). Virus can be detected, not only in the brain and other nervous tissues but also in hepatocytes, Leydig cells in the testes and epithelial cells of the respiratory and urinary tracts. Shrews express large amounts of virus in their oral epithelial cells, as well as skin keratinocytes (Durrwald *et al.*, 2014). Infectious virus and viral RNA can be demonstrated in saliva, urine, skin, tears and feces (Nobach *et al.*, 2015). There is also evidence for a wild reservoir of BoDV-1 in bank voles (*Myodes glareolus*) in northern Europe (Kinnunen *et al.*, 2007, 2013).

Horses

Naturally occurring Borna disease has been recognized in horses in eastern Germany since the 18th Century. However, it gained notoriety (and its name) when in 1894–1896 it immobilized a regiment of cavalry based in the town of Borna in Saxony (Richt *et al.*, 2000). Equine BoDV-1 infection occurs naturally only in Germany, Switzerland, Lichtenstein and Austria where on average about 12% of horses are seropositive.

The typical disease course in horses is characterized by an acute encephalitis that develops following an incubation period of 4 weeks to 3 months (Richt *et al.*, 1997). Non-specific clinical findings such as depression (apathy, somnolence, stupor), fever and anorexia precede ataxia. Horses spread their legs or cross them, and support themselves by pressing their head against the manger or wall. Repetitive behaviors including circling, vacuous slow motion chewing and severe tooth grinding may occur. Eventually, the horses become paretic, develop neurogenic torticollis, or blindness, coma and death. Lethality exceeds 80% (Ludwig and Bode, 2000; Lipkin *et al.*, 2011). Death usually occurs 1–4 weeks after the onset of clinical signs. A chronic recurring form of the disease may develop in up to 10% of cases and thus some horses may become persistently infected carriers. Serologic surveys indicate that infection without obvious clinical disease is common (Richt *et al.*, 2000).

On necropsy, horses show no gross lesions. Histopathology shows a non-purulent meningoencephalomyelitis with randomly scattered inflammatory foci in the hippocampus and along the central axis including the mesencephalon and the hypothalamus (Fig. 2). These foci consist of extensive lymphocytic perivascular infiltrates involving primarily the gray matter (Rott and Becht, 1995; Bilzer *et al.*, 1996). Most of the inflammatory cells in these foci are CD3+ T cells (Caplazi and Ehrensperger, 1998). Of these, CD4+ cells outnumber CD8+ T cells. Natural killer (NK) cells are likely present in the infiltrates as well (Hatalski *et al.*, 1998). Macrophages are fewer and B cells/plasma cells fewer still. Polymorphonuclear leukocytes are rarely present. The highest viral titers occur in the hippocampus and the piriform cortex reflecting viral invasion from the olfactory bulbs. The lowest viral titers are in the cerebellum (Gosztonyi, 2008). The virus is primarily located within neuronal nuclei (Richt *et al.*, 2000). Less consistently, smaller amounts of viral antigen may be detected in the neuronal perikaryon, dendrites and axons (Bilzer *et al.*, 1996). There is usually a correlation between viral titer and the severity of the brain lesions and the inflammatory exudate is associated with the presence of viral antigen, but the inflammatory cells never appear to contain viral

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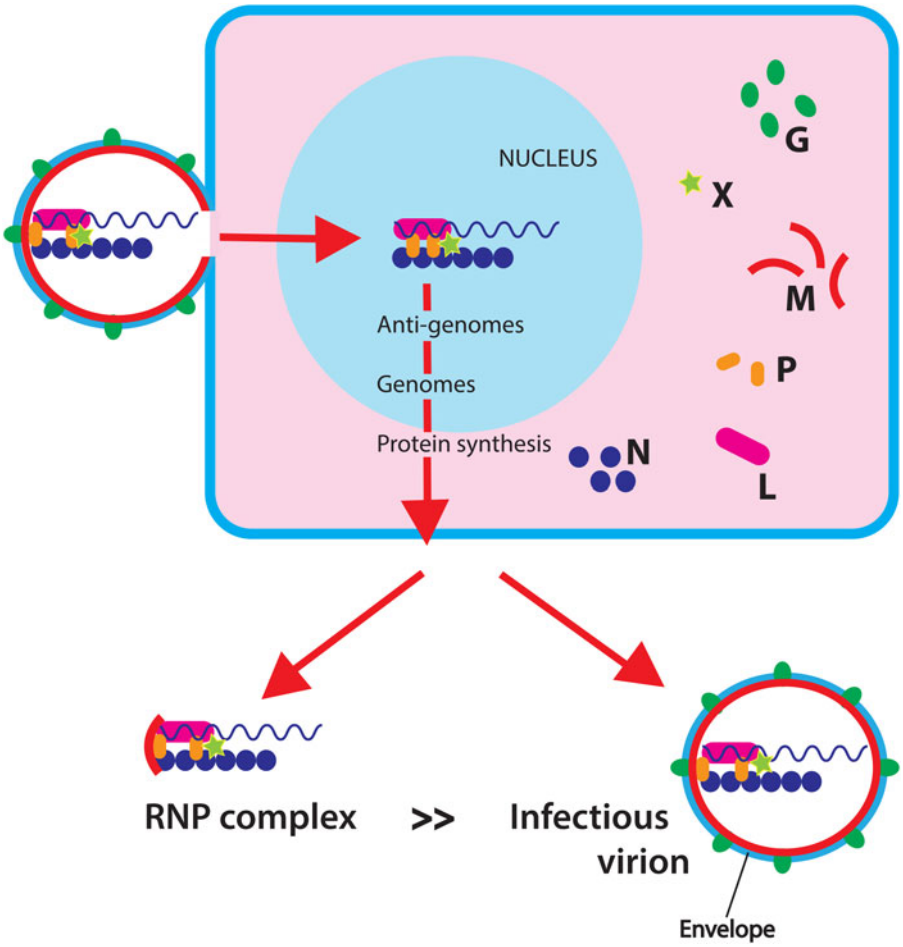

Fig. 1. The intranuclear replication of bornaviruses. The viral ribonucleoprotein (RNP) complex is imported into the nucleus. It binds to the nuclear chromatin and generates more of these RNP complexes. The virus rarely forms complete virions and as a result, it probably spreads between cells in the form of the RNP.

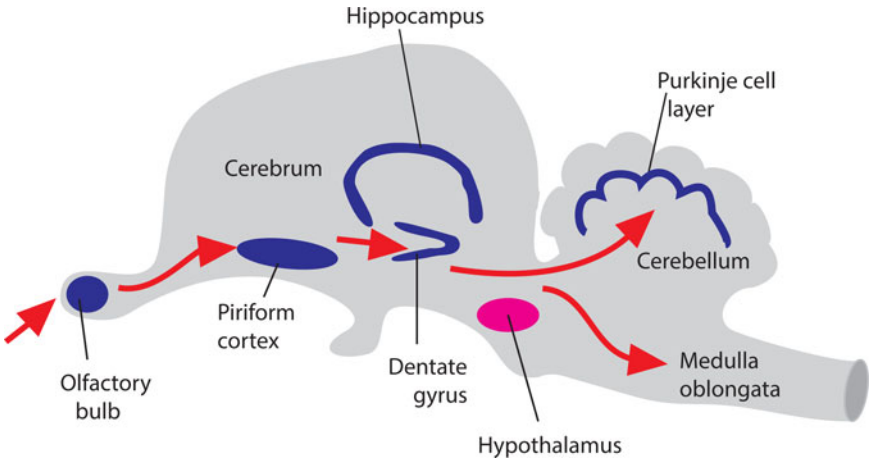

Fig. 2. The major sites within the equine brain that BoDV-1 appears to favor. Infected neurons are also scattered diffusely throughout the cerebrum. The arrows indicate what is believed to be the 'natural' route of viral invasion, originating in the olfactory bulb.

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antigens (Gosztonyi and Ludwig, 1984). In chronic infections, viral antigen is demonstrable in astrocytes and the meningeal surface is infiltrated with mononuclear cells (Richt *et al.*, 2000). Inflammation may spread from the gray matter into the adjacent white matter and involve nerve roots and the spinal ganglia (Johnson, 1980).

Sheep

Natural Borna disease in sheep resembles that in horses although large numbers of sheep may be affected within a flock unlike horses where the disease usually affects few animals (Richt *et al.*, 1997). Clinical signs vary from minor behavioral changes to severe encephalomyelitis reflecting the intensity of the inflammatory response in the brain (Vahlenkamp *et al.*, 2002). A short period of depression precedes overt disease including somnolence, ataxia and multiple deficits (Metzler *et al.*, 1976; Waelchli *et al.*, 1985). As in horses, the majority of BoDV-1 infections in sheep remain asymptomatic. The location of the virus within the brain is identical to that in horses. Lethality in clinically affected animals is at least 50%. Some sheep recover completely while others survive but fail to thrive (Metzler *et al.*, 1979).

Cats

BoDV-1 causes staggering disease in cats (Lundgren *et al.*, 1995b; Wensman *et al.*, 2014; Lutz *et al.*, 2015). This begins with fever, apathy and a reduced appetite. Cats eventually develop ataxia, gait disturbances, blindness, lower back pain, behavioral changes, loss of postural reactions and hind-leg paralysis (Wensman *et al.*, 2014). Some may be unable to retract their claws (Lutz *et al.*, 2015). A similar disease can be induced by experimental challenge with BoDV-1 (Lundgren *et al.*, 1997). Some cats recover. There are anecdotal reports of cats surviving the acute infection and later developing extreme obesity (Wensman *et al.*, 2014). The lesions in the cat brain are similar to those observed in horses although plasma cells may be more prominent (Lundgren *et al.*, 1997). While it is possible that cats become infected by eating small infected mammals or birds (Berg *et al.*, 2001) it is just as likely that it results from exposure to the urine of infected shrews (see below). It has been suggested that as in horses and sheep, the virus probably enters the cat through the olfactory epithelium and the oropharyngeal mucosa and then gains access to the brain by intraaxonal spread (Wensman *et al.*, 2014).

BoDV-1 triggers an intense T cell reaction in the cat brain. Lundgren *et al.* observed elevated T cell numbers in the blood and brains of experimentally challenged cats (Lundgren *et al.*, 1995a, 1997). Berg *et al.* used flow cytometry to demonstrate that the CD8+ T cells in the brain of infected cats belong to a non-major histocompatibility complex (MHC) restricted population and suggested that they could be important in viral clearance from neurons (Berg *et al.*, 1999). Some cats may develop disease in the absence of gross encephalitis, a disease process similar to that seen in neonatal rats (See below). Infected cats express high levels of interferon (IFN)-γ in their brains and this may promote NK and T cell cytotoxicity and neuronal destruction (Wensman *et al.*, 2011, 2014). However BoDV-1 can survive in the presence of IFN-γ (Wensman *et al.*, 2014). CD8+ cells stimulated by BoDV-1 are found in blood, spleen and brain. Cats may also develop inflammatory changes in their intra-abdominal ganglia and the adrenal medulla (Wensman *et al.*, 2012).

Humans

At least one bornaviral species can infect humans. In 2011–2013, three breeders of variegated squirrels (*Sciurus variegatoides*) in Germany developed a progressive meningoencephalitis and died within 2–4 months (Hoffmann *et al.*, 2015). Their clinical disease progressed from a fever, to progressive psychomotor slowing, confusion, ocular paresis, coma and death. On autopsy, their brains showed edema, gliosis, lymphocyte infiltration with perivascular cuffing, and necrosis. Subsequently a bornavirus was isolated from their brains as well as from the brains of one of their squirrels. Sequencing of this virus genome revealed that it was a previously unidentified mammalian bornavirus (VSBV-1). The distribution of virus in the brains of these patients and in the squirrel resembled that seen in equine Borna disease. There was a high viral RNA load in an oropharyngeal swab from the squirrel suggesting that squirrels may act as carriers and that the virus may have been accidentally transmitted by squirrel bites (Hoffmann *et al.*, 2015). One of these patients had high levels of autoantibodies to the Yo autoantigen, an antigen located within Purkinje cells. However other authors have considered the presence of these autoantibodies in other viral encephalitides to be a clinically irrelevant epiphenomenon (Jarius and Wildemann, 2015).

In 1985, it was suggested that Borna disease virus could cause mental illness in humans (Amsterdam *et al.*, 1985). Thus Rott *et al.* using an indirect immunofluorescent focus assay, detected antibodies to BoDV-1 in 16 out of 979 psychiatric patients but none were found in 200 normal volunteers (Rott *et al.*, 1985). Fu *et al.* also reported that patients with mental illness had antibody titers against BoDV-1 (Fu *et al.*, 1993). These, and subsequent reports stimulated extensive investigations into possible links between bornaviral infection and mental health. It was suggested that BoDV-1 was associated with human neuropsychiatric diseases including bipolar disorder, chronic fatigue syndrome, schizophrenia and unipolar depression. However, isolation of BoDV-1 from humans is rare, and the serologic results obtained are possibly mis- or over-interpreted (Lipkin *et al.*, 2011). Positive reverse-transcriptase polymerase chain reaction results may have been a result of inadvertent laboratory contamination, because the human-derived sequences showed marked similarity to animal-derived laboratory strains (Durrwald *et al.*, 2007). It is now generally accepted that bornaviruses are not a significant cause of human neuropsychiatric disease (Lipkin *et al.*, 2011; Hornig *et al.*, 2012).

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Rodents

Experimental BoDV-1 infection of laboratory rats and mice has revealed many key features of bornaviral pathogenesis. One of the most significant features of these infections is the difference between the nature of the disease in adult and newborn rats.

Adult rats

Experimental infection of immunocompetent adult rats with BoDV-1 results in the development of an encephalitis similar to that observed in horses and sheep. The lesions that develop depend on the age and immune status of animals as well as their genetic background, the route of inoculation and on the passage number of the virus (Wu *et al.*, 2013). Many infected adult rats die within 1–4 months as a result of the encephalitis but 50–80% may survive and develop behavioral abnormalities or an obesity syndrome (Hirano *et al.*, 1983; Narayan *et al.*, 1983a).

BoDV-1-inoculated adult rats develop illness after an incubation period of 17–90 days, the time required for the virus to spread in dendritic-axonal processes from the inoculation site to the hippocampus (Carbone *et al.*, 1987). Intranasal inoculation of virus results in spread to the olfactory bulb by 4–6 days, and to the rest of the brain in 20 days. Inoculation into the footpads results in intraaxonal spread towards the brain. The virus then migrates from the dorsal root ganglia adjacent to the lumbar spinal cord, to the gracilis nucleus in the medulla, the pyramidal cells in the hippocampus and eventually, results in clinical disease. This progression takes 50–60 days (Carbone *et al.*, 1987). Intravenous inoculation of rat foot veins fails to cause infection suggesting that the virus does not cross the vascular endothelium (Carbone *et al.*, 2001). Sectioning of the foot nerve within 1 day of footpad inoculation also prevents viral migration to the brain but not if the nerve is cut later. Once introduced into the rat central nervous system, BoDV-1 persists in the brain and spinal cord (Herzog *et al.*, 1984). In immunocompetent adult rats neither infectious virus nor viral antigens can be detected in lung, spleen, kidney, muscle, peritoneal macrophages or blood leukocytes (Stitz *et al.*, 2002).

Infected adult Lewis rats show increased alertness at 20 days post-infection. Eventually this develops into frenzied behavior (exaggerated motor responses to minor stimuli), aggression and ataxia (Narayan *et al.*, 1983a). The onset of these behaviors coincides with the development of encephalitis and retinitis that reaches maximum intensity 30–40 days after infection. The cessation of this active phase coincides with a decline in inflammation and the onset of blindness and results in a change to a passive behavior with apathy, somnolence and depression. The diminution of inflammation may be associated with the development of static hydrocephalus. By 200 days, there is much virus but minimal inflammation in the brain (Narayan *et al.*, 1983b).

Neonatal rats

In neonatal rats, experimental infection with BoDV-1 results in transient mild inflammation, and there are no immediate clinical signs of disease. However the virus does cause glial activation that eventually leads to significant changes in brain development, behavioral abnormalities and a life-long persistent infection (Hornig *et al.*, 1999). This infection is initiated at a time when the rat brain is continuing to develop and its neuronal connections are being tuned and adapted to environmental influences (Gonzalez-Dunia *et al.*, 2005). Herzog *et al.* compared BoDV-1 distribution following challenge in adult and neonatal rats. In adults, the virus was restricted to the central nervous system, but in neonates it was also found in the heart, adrenal, stomach and intestine but not blood (Herzog *et al.*, 1984).

This persistent infection of newborn rats results in selective injury to those areas of the brain undergoing significant postnatal development (de la Torre, 2002). Thus, there is neuronal loss in the cortex, hippocampus and cerebellum (Fig. 3). The lesions in the hippocampus are concentrated in the granule cells of the dentate gyrus. This neuronal degeneration results from progressive granule cell apoptosis (Hornig *et al.*, 1999). Neonatal neuronal plasticity is accompanied by formation of new synapses and an increase in dendritic arborization (Engert and Bonhoeffer, 1999). Synaptic density is altered in BoDV-1-infected neonatal rats and in BoDV-1-P transgenic mice (Gonzalez-Dunia *et al.*, 2000; Kamitani *et al.*, 2003)

Eisenman *et al.* showed reduced cerebellar size but normal lamellar organization in BoDV-1-infected neonatal rats (Eisenman *et al.*, 1999). Gaps eventually develop in the Purkinje cell layer of the cerebellum and it has been estimated that up to 75% of Purkinje cells may be lost in these animals (Bautista *et al.*, 1995; Eisenman *et al.*, 1999).

Persistently infected neonatal rats also develop an astrocytosis (Ovanesov *et al.*, 2008a). These astrocytes control homeostasis around synapses and have a key role in removing excess neurotransmitters such as glutamate (Coulter and Eid, 2012). Bornavirus-infected astrocytes have a reduced ability to take up glutamate (de la Torre, 2002) (see below).

Persistently infected neonatal rats have impaired cognitive functions, and deficiencies in fear conditioning (Carbone *et al.*, 2001; Pletnikov *et al.*, 2002). Despite their cerebellar abnormalities, they do not show ataxia, but they do exhibit novelty-induced hyperactivity, chronic anxiety and have abnormal sleep-wake cycles, as well as decreased play behavior (Hornig *et al.*, 2001; Pletnikov *et al.*, 2002). These neonatal rat behaviors resembles some human neuropsychiatric disorders especially autism spectrum disorder (Lancaster *et al.*, 2007).

Mice

The results of experimental BoDV-1 infection in adult mice are strain specific (Rubin *et al.*, 1993). It may result in non-symptomatic infection (Hallensleben *et al.*, 1998) or fatal encephalitis depending on the strain of mice used (Hausmann *et al.*, 1999). For example, 13% of infected C57BL/6 mice show mild transient symptoms. In contrast, 80% of infected MRL mice develop severe disease although the amounts of virus in their brains are comparable (Rubin *et al.*, 1993). Intracerebral inoculation of BoDV-1 into β2-Microglobulin-deficient (CD8-) newborn

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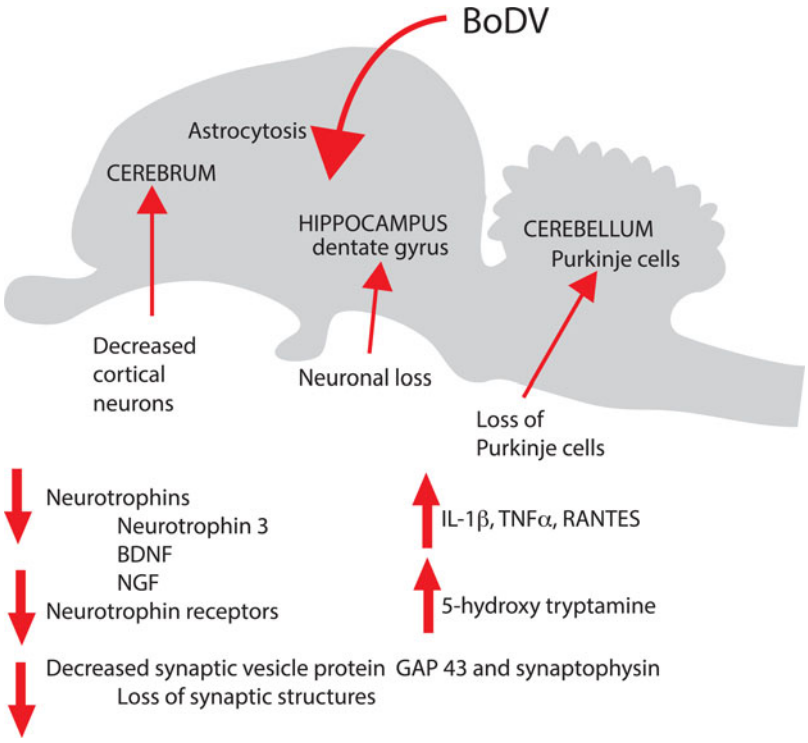

Fig. 3. A schematic diagram showing the multiple alterations in neurogenesis induced by BoDV-1 in neonatal mice.

mice of both strains does not result in disease (Hallensleben *et al.*, 1998).

Ackermann *et al.* generated a mouse-adapted BoDV-1 that expressed green fluorescent protein, and then infected mice intracerebrally. By 28 days after challenge, labeled astrocytes were detected in the lower hippocampus (the subiculum). Eventually infected neurons were found throughout the hippocampus. The virus was found in Purkinje cells and in the inner granule layer of the cerebellum by day 65. The virus was also expressed in cerebral neurons and from day 65 onward was found in the neurons of the spinal cord. By day 120 the virus was detectable in the sciatic nerve (Ackermann *et al.*, 2010). BoDV-1 infection of neonatal mice causes neurologic disease 4–6 weeks after intracerebral infection. The animals show abnormal hind leg positioning and eventual paraparesis (Narayan *et al.*, 1983a).

Gerbils

Experimental BoDV-1 infection of neonatal gerbils (*Meriones unguiculatus*), unlike neonatal rats, resulted in their death within 30 days. Virus was readily detected in their brains associated with the development of acute inflammatory lesions (Nakamura *et al.*, 1999). Despite severe symptoms and high levels of virus, there was no apparent neuronal loss (Watanabe *et al.*, 2001). In gerbils that had not sickened, the virus was detected in the cerebral cortex and the hippocampus. As disease progressed, viral expression increased in the lower brain stem and cerebellum, especially within Purkinje cells (Watanabe *et al.*, 2001). However while infected newborn gerbils developed fatal neurologic disease those infected 14 days after birth survived (Lee *et al.*, 2003). Very low levels of virus were detected in their brains. Additionally, neonatal gerbils treated with cyclosporine A were not protected against fatal disease. The cyclosporine did however prevent brain inflammation and significantly reduced brain cytokines (except interleukin (IL)-1β) (Watanabe *et al.*, 2003).

Other species

BoDV-1 has been used to induce experimental infections in many other mammals. The clinical disease and outcome vary between species. Rabbits, for example, develop a fatal paralytic disease similar to that seen in horses (Richt and Rott, 2001); tree shrews (*Tupaia glis*) develop aberrant neurologic behaviors (Richt *et al.*, 1992); and rhesus monkeys develop severe paralytic disease with retinopathy (Stitz *et al.*, 1981; Richt *et al.*, 1992). BoDV-1 has been detected in two dogs with neurologic symptoms (Weissenbock *et al.*, 1998; Okamoto *et al.*, 2002). Cattle can also develop Borna disease but it is uncommon and sporadic (Bode *et al.*, 1994; Caplazi *et al.*, 1994). Bornaviral encephalitis has also been recorded in captive alpacas and wild deer (Jacobsen *et al.*, 2010).

The pathogeneses of Borna diseases

When viruses invade the brain a consistent defensive response is mounted. Thus virus-infected cells are detected by resident

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microglia and recruited macrophages. These responding cells become activated and release a mixture of antiviral cytokines and chemokines (Russo and McGavern, 2015). These cytokines in turn, attract T cells, NK cells and other mononuclear cells to the site of invasion. Antigen presentation by the antigen-processing cells, release of cytokines and other mediators from microglia and astrocytes stimulates a type 1 antiviral T cell response (Hatalski *et al.*, 1998). For many, but not all viruses, this T cell response is sufficient to eliminate the virus. This is not the case in Borna diseases.

Encephalitis

In BoDV-1 infected rats, the most severely affected brain regions include the olfactory bulb, the dentate gyrus, the caudate nucleus and the hippocampus as well as adjacent structures such as the mesencephalon, central gray matter, substantia nigra and hypothalamus (Tomonaga *et al.*, 2002). BoDV-1 thus exhibits a preferential tropism for the rodent limbic system (de la Torre, 2002). Unfortunately, the viral cellular receptor(s) have yet to be identified, so the biochemical basis of this tropism is unknown.

BoDV-1 enters cells by receptor-mediated endocytosis (Gonzalez-Dunia *et al.*, 1998). The primary route of natural invasion in most mammals is most likely through the nasal epithelium (Morales *et al.*, 1988; Sauder and Staeheli, 2003). The olfactory bulbs of experimentally infected horses show inflammation and edema early in disease (Solbrig and Koob, 2003). The virus replicates in the neurons at the initial entry site and then migrates intra-axonally towards the brain (Carbone *et al.*, 1987; Salinas *et al.*, 2010). The viral surface glycoprotein is required for cell to cell transfer (Bajramovic *et al.*, 2003; Lennartz *et al.*, 2016). The viral RNP complex spreads by axonal and polysynaptic neuronal transmission. Once within the axon, intracellular microtubules transport the viral RNP (Clemente *et al.*, 2010). The protein dynein forms motor complexes with BoDV-1 RNP and transport it from the nerve terminal to the cell body (Gosztonyi *et al.*, 1993; Clemente *et al.*, 2010). Subsequently, RNP spreads to other cells such as astrocytes, oligodendroglia, ependymal cells and possibly Schwann cells. Eventually viral RNA can be detected in all peripheral nerves (Enbergs *et al.*, 2001). As inflammation develops, perivascular cuffs form. These cuffs contain CD4+ and CD8+ T cells and macrophages (Hatalski *et al.*, 1998).

BoDV-1 is not cytotoxic in cultured cells (Stitz *et al.*, 2002; Matsumoto *et al.*, 2012). The virus is strongly cell associated and produces very few infectious virions per cell even though large quantities of RNP are present (de la Torre, 2002; Tomonaga *et al.*, 2002). Virions are not detected in infected brain either, suggesting that virus spreads as an RNP complex (Zimmermann *et al.*, 1994). Transgenic mice expressing BoDV P-protein in their astrocytes show major behavioral abnormalities such as aggressiveness, hyperexcitability and special reference memory deficit resembling those seen in bornavirus-infected mice (Honda *et al.*, 2011). These behaviors are associated with alterations in the expression of genes associated with the transforming growth factor (TGF)-β pathway (Kamitani *et al.*, 2001; Nishino *et al.*, 2015).

Wu *et al.* infected hippocampal slice cultures of several rat strains with BoDV-1 (Wu *et al.*, 2013). Cultures from some strains such as Lewis (LEW) showed disrupted architecture while others, such as Sprague Dawley (SD) did not. The efficacy of viral replication was however identical in cultures from different rat strains. These strain differences also occur in vivo. Media harvested from uninfected LEW or SD cultures could prevent BoDV-1-induced damage in LEW cultures. Infection with BoDV-1 reduced the availability of this inhibitory factor in LEW but not SD cultures. Genetic analysis indicated that a bornaviral resistance locus is present on rat chr6q16 and a susceptibility locus on chr3q21–23 (Wu *et al.*, 2013).

Retinitis

BoDV-1 spreads to the retina from the brain along the optic nerve. Krey *et al.* blocked the optic nerves of rabbits by xenon coagulation and then infected them intracerebrally with BoDV-1 (Krey *et al.*, 1979). Retinopathy did not develop, and viral antigen could not be detected in animals with blocked nerves. Blindness is regularly observed in equine Borna disease as a result of retinal degeneration with lymphoplasmacytic infiltration (Bilzer *et al.*, 1996). Lymphocytic infiltrates may also be observed within the optic nerve. There is a great diversity in its severity, and not all horses have detectable bornavirus in their retinas (Dietzel *et al.*, 2007).

Muller cells are retinal glial cells (Kacza *et al.*, 2000, 2001). The neuron:Muller cell ratio in the retina is significantly reduced in diseased horses as compared with controls. This appears to be due to a concomitant loss of neurons and an increase in glia. The neuronal degeneration begins in the outer retinal layer where the photoreceptors are located but all retinal layers show reduced thickness (Kacza *et al.*, 2000).

Neurons are also lost in the retinas of BoDV-1 infected rats and rabbits (Narayan *et al.*, 1983b). Four weeks after intracerebral inoculation of rats there is a significant thinning of the retina due to a loss of photoreceptor segments and ganglion cells. At the same time, there is a great increase in the number of glial cells in the ganglion cell and inner plexiform layers (Kacza *et al.*, 2000). Microglia and macrophages are involved in the neuronophagocytosis that accompanies this neurodegeneration (Kacza *et al.*, 2000). Muller cells show moderate changes (Iandiev *et al.*, 2006). Kacza *et al.* infected Lewis rats with BoDV-1 intracerebrally (Kacza *et al.*, 2001). Within months their retinal thickness had declined to a third of that in control animals. Photoreceptor segments were completely destroyed and the number of neurons reduced. There were many active microglia and macrophages undertaking neuronophagocytosis. Muller cells showed signs of gliosis, alterations in glutamate synthetase, altered K^+ currents and thickened stem processes (Kacza *et al.*, 2001).

Narayan *et al.* attributed bornaviral retinitis to a transient attack by cytotoxic T cells (Narayan *et al.*, 1983b). For example, Krey *et al.* treated BoDV-1 infected rabbits with

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immunosuppressive drugs that delayed the onset of retinitis (Krey *et al.*, 1981). The treatment reduced the confluency of the retinal lesions and some treated animals either lacked eye lesions or showed nonprogression. Subsequently, Stahl *et al.* showed that the retinal T cell infiltration consisted of αβTCR+, CD4+, CD8+ cells (Stahl *et al.*, 2003). B cells were rarely found by Stahl *et al.* but Hatalski *et al.* found them to be plentiful (Hatalski *et al.*, 1998). This may reflect the use of different reagents. Cytokine transcripts in affected retinas included raised IL-1β, IL-6, IFNγ and tumor necrosis factor (TNF)-α, as well as CXCL10, chemokine ligand (CCL)2 and CCL4. By day 36 the levels of these transcripts had returned to normal. These cytokines were probably derived from the infiltrating T cells and NK cells (Sauder and de la Torre, 1999). Chronic bornaviral infections may result from a switch in the brain-infiltrating T cells from generating a Th1 response to a Th2 response (Hatalski *et al.*, 1998).

Alterations in neurogenesis

In the developing rodent brain, neurogenesis occurs predominantly in the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus (Katsumoto *et al.*, 2014). It is unclear how neuronal loss occurs in persistently infected neonatal rats but it is probably due to induced apoptosis (Hornig *et al.*, 1999; Zocher *et al.*, 2000; Ovanesov *et al.*, 2008b). Damage to synaptic structures precedes neuronal loss in persistently infected rats and this may impair trafficking of growth factors (Volmer *et al.*, 2007). Bornaviral-induced disturbances in the neurotrophin system also contribute to this neurodegeneration. Thus Zocher *et al.* found reduced levels of neurotrophin 3, brain-derived neurotropic factor (BDNF) and nerve growth factor in the hippocampus 14-days post-infection in newborn rats and in the cerebellum by 21 days post-infection (Zocher *et al.*, 2000) (Fig. 3). They also detected reduced levels of neurotrophin receptors in both the hippocampus and cerebellum. Neonatal persistently-infected rats fail to gain weight but lack of nourishment does not account for the brain lesions or the lack of weight gain (Dietz and Pletnikov, 2003). There are increased concentrations of serotonin in the hippocampus of bornavirus- infected neonates (Dietz and Pletnikov, 2003). Hans *et al.* examined the effect of bornaviral infection on the response of hippocampal neurons to neurotrophin BDNF. Persistent infection blocked BDNF-induced extracellular signal-regulated kinases-1/2 (ERK) phosphorylation even although the expression of the BDNF receptor was normal (Hans *et al.*, 2004). As a result, BDNF-induced expression of synaptic vesicle proteins is blocked, potentially causing defective synaptic organization. Persistently-infected newborn rats show a progressive decline in the expression of synaptic markers (growth-associated protein 43, a presynaptic membrane phosphoprotein and synaptophysin, a calcium-binding protein found in presynaptic vesicles) followed by a loss of up to 30% of cortical neurons (Gonzalez-Dunia *et al.*, 2000). This decline in synaptic density and neuronal plasticity occurs primarily within the cerebrum and the hippocampus.

Obesity

Some adult rats that survive acute BoDV-1 disease begin to overeat and as a result, become obese ((Narayan *et al.*, 1983a; Wensman *et al.*, 2014). Obesity also develops in some persistently infected neonatal rats (Lyons *et al.*, 2002). This uncontrolled appetite may result from damage to hunger control centers in the brain (Nagashima *et al.*, 1992; Gosztonyi and Ludwig, 1995). The neonatal rats develop inflammation in their pituitary stalk (Gosztonyi and Ludwig, 1995). As the obesity syndrome develops, the number of virus-infected cells grows and leads to progressive involution of the hypothalamus as well as vacuolar degeneration of neurons in the hypothalamic paraventricular nucleus. Herden *et al.* compared the brain lesions of a pathogenic BoDV-1 strain with that of an obesity-inducing strain (Herden *et al.*, 2000). The obesity-inducing-strain lesions were restricted to the septum, hippocampus, ventromedial hypothalamus and amygdala. Herden *et al.* also examined the levels of neuropeptides in BoDV-1-infected brains and found that expression of melanocyte-stimulating hormone (MSH) was reduced in infected animals (Herden *et al.*, 2000; Herden *et al.*, 2005). α-MSH reduces appetite so its deficiency may have an opposite effect and bornaviral obesity could therefore be due to lesions in the melanocortin feeding center within the hypothalamus.

Pathogenic processes

Three major pathogenic processes collectively cause the neuronal damage associated with bornaviral encephalitis (Fig. 4). Initially, the virus replicates almost exclusively in neurons (Gosztonyi and Ludwig, 1995). This triggers immune-mediated attack by virus specific CD8+ T cells directed against viral antigens such as bornaviral N-protein or autoimmune attack against neoantigens on the neuronal surface (Amor *et al.*, 2014). In the later stages of infection, the virus infects microglia and astrocytes (Carbone *et al.*, 1991; Gosztonyi and Ludwig, 1995). These plus the results of neuronal destruction leads to the prolonged activation of microglia and a consequent increase in tissue cytokines and reactive nitrogen species (Gonzalez *et al.*, 2014). Thirdly, astrocyte dysfunction due to viral invasion disrupts glutamate regulation. Excess glutamate generated as a result kills neurons through a process called excitotoxicity.

Immune-mediated T cell attack

The primary mechanism of neuronal damage in bornaviral encephalitis is T cell-mediated neuronal cytotoxicity. These T cells are activated by viral infection and as a result, mount a type 1 response characterized by the production of both IFN-γ and TNF-α (Baruch and Schwartz, 2013). The neurotransmitters glutamine and acetylcholine also favor T cell activation (Pacheco *et al.*, 2010; Pikor *et al.*, 2015) as do activated Th17 cells (Nouri *et al.*, 2014).

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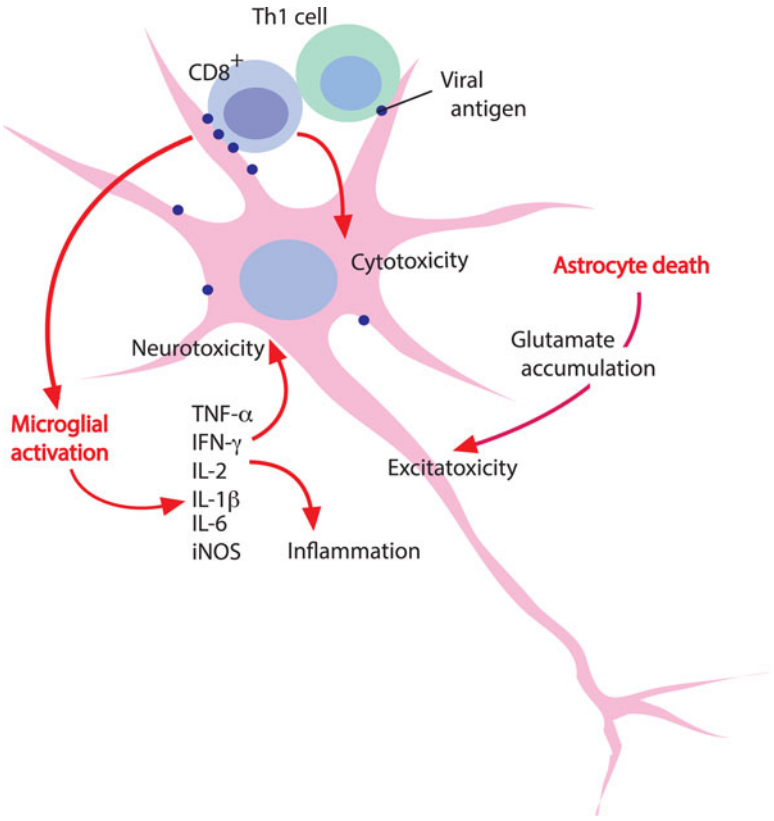

Fig. 4. The three major mechanisms of neuronal destruction mediated by BoDV-1. These are, T-cell-mediated cytotoxicity, microglial activation resulting in cytokine-mediated neurotoxicity, and loss of astrocyte function resulting in glutamate accumulation and excitotoxic destructions of neurons.

In bornaviral lesions, the perivascular cellular infiltrates predominantly consist of CD4+ T cells while CD8+ cells predominate within the brain parenchyma. The development of neuronal lesions is specifically associated with invasion by CD8+ cells (Sobbe *et al.*, 1997). The importance of these T cell infiltrates can be demonstrated by adoptive transfer of lymphocytes from infected to uninfected rats. For example, Narayan *et al.* could transmit the disease using spleen cells from 4-week-old rats transferred into cyclosporine-treated recipients (Narayan *et al.*, 1983a). Rott *et al.* were able to establish a virus-specific, CD4+ T cell line that induced typical bornaviral lesions when administered to recipient rats of the corresponding MHC class II haplotype (Rott *et al.*, 1988). Sobbe *et al.* induced typical bornaviral brain lesions in rats by adoptive transfer of CD8+ brain T cells (Sobbe *et al.*, 1997). Additionally, lymphocytes obtained from rats early in bornaviral infection could transfer the disease but not late in infection implying that the cytotoxic cells were generated early in the disease process (Narayan *et al.*, 1983a). Planz *et al.* identified a peptide, ASYAQMTTY, from BoDV-1 nucleoprotein that was recognized by CD8+ T cells in association with the rat MHC class I molecule, RT1.A, and, as a result, triggered T cell cytotoxicity (Planz *et al.*, 2001). Transgenic mice expressing the nucleoprotein are resistant to disease, presumably because they are immunologically tolerant to it (Schwemmle *et al.*, 1998). Lymphocytes from the brains of rats with acute bornaviral disease show MHC class I restricted cytotoxic T cell activity. MHC class I expression occurs on astrocytes and on some neurons in BoDV-1-infected brains. MHC class I production by neurons is normally minimal but can be induced (Planz *et al.*, 1993).

Support for the essential role of T cells is also provided by immunosuppressive studies. For example, Rott *et al.* demonstrated that T cell elimination prevented inflammation and the development of clinical disease. A single intraperitoneal injection of cyclophosphamide given to adult rats prevented the development of bornaviral encephalitis and clinical disease. Stitz *et al.* were able to prevent the development of bornaviral disease by administering cyclosporine prior to infection (Stitz *et al.*, 1989). Stitz *et al.* also treated bornavirus-infected adult rats with monoclonal antibodies against N-protein-specific CD4+ and CD8+ T cells (Stitz *et al.*, 1992). Both types of antibody suppressed the encephalitis but anti-CD8 was more effective than anti-CD4. These monoclonal antibodies worked best if given before or shortly after infection and they did not prevent encephalitis or disease when given more than 4 days after infection. BoDV-1 infection will not result in disease in athymic nude rats (Herzog *et al.*, 1985). Thus the lesions of bornaviral encephalitis in rats are mediated primarily by N-protein-specific CD8+ T cells.

In persistently infected neonatal rats, unlike adult rats, T cells may be protective. Thus persistent BoDV-1 infection could be prevented by prior administration of a virus-specific CD4+ T

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cell line. Recipient rats developed a transient, mild encephalitis that lasted for only a few days. This T cell line had no cytotoxic properties but virus clearance was accompanied by the appearance of CD8+ cytotoxic T cells in the recipients (Noske *et al.*, 1998). It is interesting to note however, that in adult rats infected with BoDV-1, the virus is found exclusively in the brain. In cyclosporine-treated rats the virus can be detected in peripheral nerve fibers and adjacent tissues such as lung, liver and spleen (Stitz *et al.*, 1991). This suggests that T cells are responsible for containing BoDV-1 within the central nervous system (CNS). In subsequent studies, Stitz *et al.* examined the role of antibodies in restricting BoDV-1 to the CNS. Immune serum transfer into cyclosporine-treated or newborn rats resulted in restriction of the virus to the CNS (Stitz *et al.*, 1998).

Hatalski *et al.* followed the evolution of the immune response in the brain of BoDV-1-infected Lewis rats (Hatalski *et al.*, 1998). Thus at the peak of the acute infection, the perivascular infiltrates contained both CD4+ and CD8+ T cells as well as a significant population of NK cells. The NK cells could be identified in the brain lesions 3.5 weeks post-infection before the onset of clinical disease. As the disease progressed and became chronic, the numbers of all cell types dropped and the authors suggested that this reflected a switch from a type 1 to a type 2 immune response. This suggestion was supported by a rise in serum IgE levels. Hatalski *et al.* also measured cytokine mRNA expression in infected brains. They showed a significant increase in the levels of the proinflammatory cytokines, IL-1α, IL-2, IL-6, TNF-α and IFN-γ that peaked at 5 weeks post-infection when acute inflammation was maximal. On the other hand, the anti-inflammatory cytokine, IL-4 increased to reach maximal levels at the end of the study at 15 weeks. The regulatory cytokine, TGF-β in contrast, peaked at 5 weeks and then stabilized (Hatalski *et al.*, 1998). It is possible that the decline in inflammation seen in long-term bornaviral infections may also be due to the development of neuroprotective Treg cells (Walsh *et al.*, 2014). Nishino *et al.* investigated this upregulation of TGF-β in BoDV-1-infected rats. They found that signal receptors for TGF-β1 were also upregulated as were inhibitin/activin βC, two components of the TGF-β pathway (Nishino *et al.*, 2009). This may reflect the ongoing immunosuppressive effects of bornaviral P-protein (Nishino *et al.*, 2015).

Microglia and the role of chronic inflammation

The second important pathogenic process in bornaviral disease results from prolonged activation of microglia (Weissenbock *et al.*, 2000; Ovanesov *et al.*, 2006, 2007, 2008a, b). In the healthy brain, microglia remodel neuronal synapses and secrete neurotrophic proteins that help maintain effective neuronal network functions (Zocher *et al.*, 2000). When activated however, microglia flood the brain with the inflammatory cytokines, TNF-α, IFN-β and IL-10 as well as induced nitric oxide synthase (iNOS), and reactive oxygen and nitrogen species (ROS and RNS) (Gonzalez *et al.*, 2014). These molecules induce neuronal dysfunction and death (Heneka *et al.*, 2014; Papageorgiou *et al.*, 2015). Zheng *et al.* have demonstrated that the severity of the neurologic signs and of the corresponding encephalitis correlated well with iNOS and cNOS mRNA expression in rat bornaviral disease and that the distribution of iNOS-positive cells in the basolateral cortex and the hippocampus correlated with sites of BoDV-1 infected cells (Zheng *et al.*, 1993). This cannot however be the whole explanation for bornaviral pathogenicity since Hausmann *et al.* demonstrated that BoDV-1 caused neurologic disease in mice that lacked IFN-γ, Fas, iNOS or the chemokine receptor, CXCR3 (Hausmann *et al.*, 2004).

Microglia are activated by BoDV-1 (Plata-Salaman *et al.*, 1999; Ovanesov *et al.*, 2006) (Fig. 5). In BoDV-1-infected adult rats, these activated microglia can be detected in the dentate gyrus at 10 days post-infection but detectable loss of granule cells is not seen until 30 days. Thus the virus activates the microglia long before neuronal loss and it is therefore unlikely that the activated microglia alone trigger neuronal loss and dysfunction (Ovanesov *et al.*, 2008b). Nevertheless, increased levels of brain IL-6, TNF-α and iNOS mRNAs do correlate with the severity of the inflammatory lesions in infected brains (de la Torre, 2002).

Classically activated microglia (M1 cells) have two alternative fates. They may differentiate into regulatory cells (M2 cells) and so reduce inflammation and promote tissue repair or alternatively, they can undergo uncontrolled activation and trigger chronic inflammation resulting in the production of neurotoxic factors and progressive neural loss (Gonzalez *et al.*, 2014). M2 activation of microglia enhances their release of neurotrophic factors, proteases, IL-4, TGFβ and arginase 1 and stimulates their phagocytic activity. M2 activation thus reduces inflammation and promotes tissue repair. The anti-inflammatory cytokines, IL-4 and TGFβ are produced during the later stages of bornaviral encephalitis (Hatalski *et al.*, 1998). While they are mainly derived from T cells, they may also be produced by microglia (Heneka *et al.*, 2014).

Morimoto *et al.* used dexamethasone to inhibit bornavirus-induced inflammation in rats. They suggested that bornaviral disease involved an early inflammatory reaction mediated by resident microglia leading to sensitization (antigen processing and presentation), and an influx of primed T cells. Restimulation of these infiltrating T cells led to the local production of a cytokine mixture amplifying the reaction. Subsequently, recruitment and activation of microglia continued this process. Morimoto *et al.* suggested that the initial expression of pro-inflammatory cytokines was directly mediated by microglial BoDV-1 (Morimoto *et al.*, 1996).

Astrocytes and glutamate toxicity

The third major mechanism of bornaviral-induced neuronal destruction results from astrocyte-mediated disturbances in glutamate levels.

Astrocytes are the major microglial cell population in the brain (Rossi and Volterra, 2009). Under normal conditions, they supply glucose to neurons and regulate the composition of extracellular fluid. Importantly, they remove excess potassium ions and neurotransmitters, especially glutamate (Coulter and Eid, 2012).

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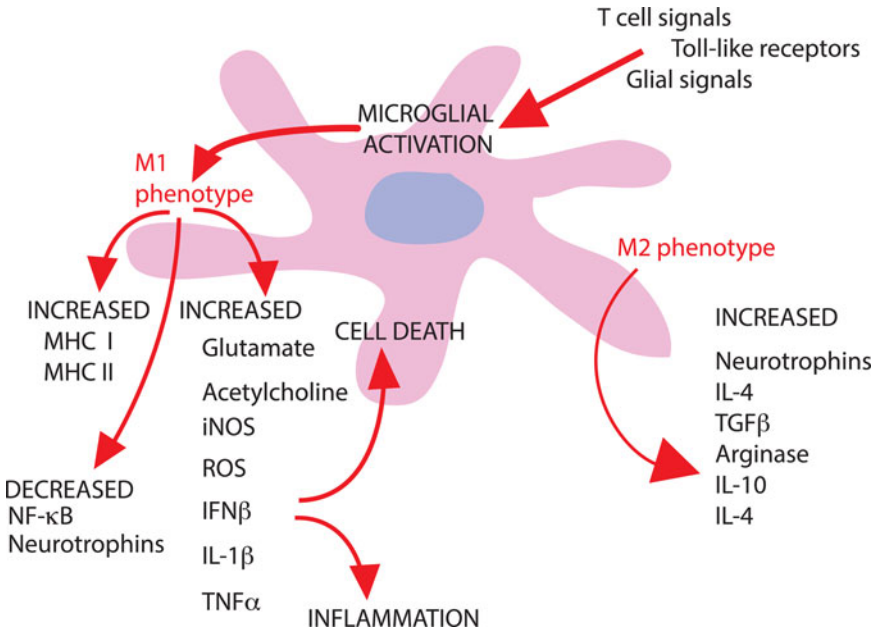

Fig. 5. The role of microglial activation in the pathogenesis of bornaviral encephalitis. M1 activation of the microglia results in the flooding of the brain with multiple cytokines as well as potent oxidants resulting in neuronal death. Should the microglial phenotype change to M2, the resulting cytokines will reduce inflammation and cell destruction and promote repair although destroyed neurons unlikely to be replaced.

Astrocytes take up extracellular glutamate, transform this into glutamine using glutamine synthetase and subsequently shuttle the glutamine back to neurons using transporters. Between 80 and 90% of extracellular glutamate uptake in the brain is through these astrocytic glutamine transporters (Vesce *et al.*, 2007; Coulter and Eid, 2012) (Fig. 6). Once within neurons glutamine is reconverted to glutamate. This glutamate may act as a neurotransmitter directly or it is decarboxylated to form γ-amino butyric acid (GABA), which also acts as a neurotransmitter.

Excitotoxicity

Excitatory synaptic transmission in the brain is mainly mediated by glutamate (Bondy and Purdy, 1977; Choi, 1988). If the extracellular concentration of glutamate is excessive, neurons will be damaged. This process is called excitotoxicity and the excessive production of glutamate within the brain is called a glutaminergic storm. Neurons die as a result of excessive influx of calcium through glutamate receptor channels leading to mitochondrial damage and the production of ROS and RNS (Gudino-Cabrera *et al.*, 2014). Excitotoxicity has been associated with neurodegeneration (Jacobs *et al.*, 2006). Excessive glutamate may also impair the blood-brain barrier (Gudino-Cabrera *et al.*, 2014).

In the healthy brain, homeostasis is maintained by the removal of excess glutamate by astrocytes. If these astrocytes are lost and replaced by microglia, the glutamine-glutamate cycle is down-regulated (Schwartz *et al.*, 2003). TNF-α upregulates the production of glutamate in activated microglia and at the same time, inhibits glutamate uptake by astrocytes (Takeuchi *et al.*, 2006).

Billaud *et al.* showed that BoDV-1 inhibits glutamate uptake by feline primary cortical astrocytes (Billaud *et al.*, 2000; de la Torre, 2002). This failure to remove glutamate could result in neuronal excitotoxicity. This is supported by the studies of Ovanesov *et al.* who measured extracellular glutamate in the striatum of Fischer 344 rats (Ovanesov *et al.*, 2007). BoDV-1 infection increased the extracellular levels of glutamate. This elevated extracellular glutamate was associated with reduced neuron numbers and volume in the striatum.

Bergmann glial cells are astrocytes that surround Purkinje cells where they sequester neuronally-produced glutamate. Purkinje cells disappear from the cerebellum of BoDV-1-infected neonatal mice although they themselves may not be infected. The Bergmann glia are however infected and it is possible that damage to these cells could result in secondary loss of Purkinje cells (Bordey and Sontheimer, 2003).

On the other hand, Richter *et al.* showed that two glutamate receptor antagonists failed to prevent bornaviral-induced neuronal loss in IFNγ-deficient mice. While there was a trend towards protection it was not statistically significant. Richter *et al.* also demonstrated that neither the frequency of virus-infected cells, nor the composition of the T cell infiltrate was altered by these antagonists and they concluded that glutamate excitotoxicity was an unlikely cause of neuronal damage (Richter *et al.*, 2009). They did not however exclude toxic effects mediated through the third glutamate receptor – kainite. The kainite receptor (KA-1) has been suggested to be a BoDV-1 receptor or target (Gosztonyi, 2008).

Zhang *et al.* analyzed metabolites in bornavirus-infected areas of the equine hippocampus (Zhang *et al.*, 2014). They found that infected tissues had lower levels of D-myo-inositol-1-phosphate, glutamate, phosphoethanolamine, heptadecanoic acid and

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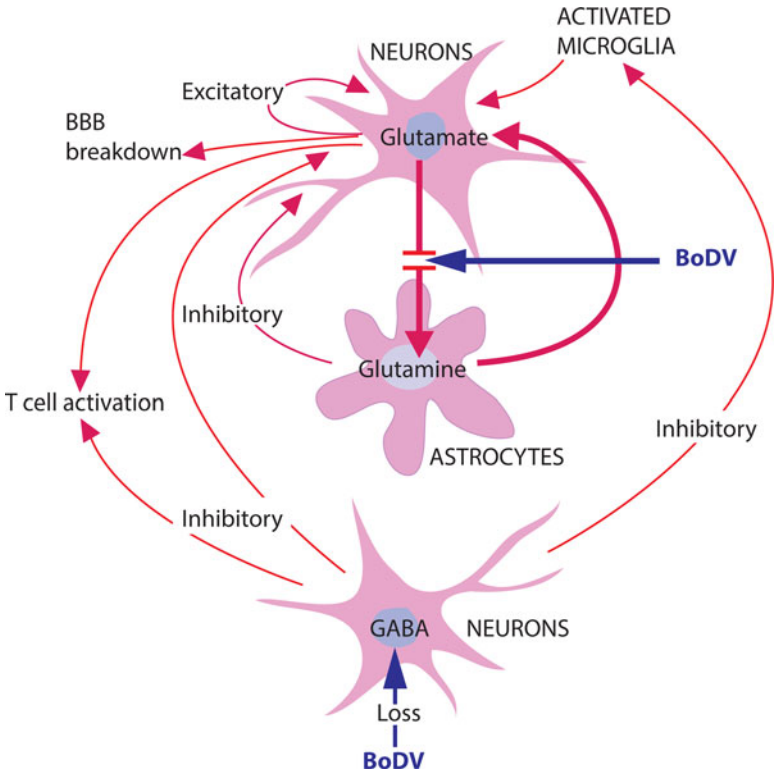

Fig. 6. The role of glutamate in BoDV-1 encephalitis. The normal glutamate cycle requires that astrocytes remove excess glutamate from the extracellular fluid, convert it to glutamine and return it to the neurons. If astrocytes are damaged glutamate accumulates and kills neurons. GABAergic neurons release GABA that inhibits glutamate excitotoxicity and microglial activation. If these neurons are also damaged by BoDV-1 then additional neuronal loss will be expected.

linoleic acid but higher levels of ammonia. These results also confirm that there are disturbances in glutamate metabolism within these tissues.

Effects of glutamate on microglia

Glutamate release by astrocytes is influenced by inflammatory mediators such as TNF-α and prostaglandins. Thus inflammation may disrupt astrocyte-neuronal interactions (Ovanesov *et al.*, 2008a; Rossi and Volterra, 2009). Activation of microglia by BoDV-1 *in vitro* requires the presence of astrocytes (Ovanesov *et al.*, 2008a). Activated microglia also release large amounts of glutamate in response to TNF-α by upregulating glutaminase (Takeuchi *et al.*, 2006).

Effects of glutamate on T cell function

T cells have receptors for glutamate and glutamate enhances T cell adhesion, chemotactic migration and proliferation (Schwartz *et al.*, 2003; Levite, 2008). Glutamate also protects them against antigen-induced apoptosis (Ganor and Levite, 2014). Thus glutamate alters surface receptor expression, and may enhance T cell cytotoxicity. It is possible therefore that elevated glutamate in sites of bornaviral invasion might attract T cells and promote T-cell-mediated brain damage. It may be relevant to note that many neurotransmitters other than glutamate also activate T cells (Levite, 2008). For example, resting T cells can also be activated by dopamine, serotonin and some neuropeptides

The role of gamma-aminobutyric acid

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter synthesized from glutamate using L- glutamic acid decarboxylase. GABA reduces neuronal excitability by acting at inhibitory synapses. GABA also acts as a growth factor for the developing brain where it stimulates neuronal branching (Chen and Kriegstein, 2015). Microglia possess GABA receptors and the GABAergic system can regulate microgial activities. These microglia in turn may regulate GABAergic transmission by neighboring neurons. T cells and dendritic cells contain glutamic acid decarboxylase and express GABA receptors. GABA also suppresses both proinflammatory cytokine production and immune cell proliferation. Thus an increase in GABA may suppress T cell function and inflammation (Peng *et al.*, 2008).

Bornaviral P-protein binds directly to GABA-receptor-associated protein (Peng *et al.*, 2008). It is then transported to the cell nuclei where it disrupts the trafficking of GABA-receptors (Scordel *et al.*, 2015). The P-protein also reduces neurogenesis, and specifically targets GABAergic neurogenesis. This results from inhibition of the production of pro-neuronal factors

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such as *ApoE, Noggin, TH and Scg10/Strathmin2*. BoDV-1 can disrupt the GABA-glutamate cycle reducing its inhibitory effect and thus enhancing excitotoxicity and T cell activity (Fig. 6). Neonatal rats persistently infected with BoDV-1 undergo a selective loss of GABAergic cortical neurons (Bautista *et al.*, 1995; Eisenman *et al.*, 1999; Gonzalez-Dunia *et al.*, 2000; Pletnikov *et al.*, 2002). It is perhaps no coincidence that aberrations in GABAergic neurogenesis have been associated with human neuropsychiatric disorders (Scordel *et al.*, 2015).

Other effects on neurotransmission

Bornaviral encephalitis in Lewis rats is associated with a decline in the cholinergic activity of the brain (Gies *et al.*, 1998, 2001). This decline appears to be due to a loss of choline acetyltransferase activity in the cerebral cortex and hippocampus and a reduction in acetylcholinesterase in these regions. These declines parallel the loss of neurons in these areas. The decline in choline acetyltransferase occurs in the pre-encephalitic stage of bornaviral infection prior to T cell infiltration into the brains (Gies *et al.*, 1998).

BoDV-1 also affects the dopamine system. Neonatal rats experimentally infected with BoDV-1 develop a hyperactive movement disorder (Solbrig *et al.*, 1996). Since locomotor activity is regulated through the dopamine system, Solbrig *et al.* examined the dopamine receptors in these rats (Solbrig *et al.*, 1996). They found that there was reduced binding to several dopamine receptors in the nucleus accumbens. The nucleus accumbens is the site where the limbic system and motor information interact. It plays a role in motivational, appetite and locomotor behaviors. Bornaviral-induced alterations have also been recorded in other neurotransmitters such as cholecystokinin and somatostatin (de la Torre, 2002).

Conclusions

The diversity of clinical manifestations of BoDV-1 infections reflect its complex pathogenicity. Even the development of acute encephalitis reflects the results of multiple disturbances in many neuronal and immune pathways. There appear to be three primary mechanisms involved in this pathology, namely T cell cytotoxicity; microglial release of inflammatory cytokines and ROS; and astrocyte-induced disturbances in glutaminergic signaling. It is unlikely however that these are the only important pathways involved in the disease process. Thus it is recognized that bornaviruses disrupt the NF-kB pathway (Makino *et al.*, 2015), the RIG-1/MAVS pathway (Reuter *et al.*, 2010) as well as HMGB-1 signaling (Kamitani *et al.*, 2001) and can cause epigenetic changes (Liu *et al.*, 2015). These effects collectively permit the virus to survive innate immune attack and contribute to the disease process. Even in the absence of acute encephalitis BoDV-1 has a profound effect on neurodevelopmental pathways in neonatal rats. This may be of relevance to neurodevelopmental disease in humans.

During the 1990s, interest in BoDV-1 peaked as a result of assertions that it was an unusually common infection in humans with neuropsychiatric disorders. This claim has been refuted. Nevertheless, given the diversity of molecular and epigenetic changes mediated by bornaviruses in mammalian brains, it is conceivable that they can induce alterations in brain function well short of the lethal encephalitis observed in squirrel breeders. It is perhaps time to reopen the investigation into possible links between bornaviral infection and mental illness.

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