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Review

Borna disease virus – Fact and fantasy

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ABSTRACT

The occasion of Brian Mahy's retirement as editor of *Virus Research* provides an opportunity to reflect on the work that led one of the authors (Lipkin) to meet him shortly after the molecular discovery and characterization of Borna disease virus in the late 1980s, and work with authors Briese and Hornig to investigate mechanisms of pathogenesis and its potential role in human disease. This article reviews the history, molecular biology, epidemiology, and pathobiology of bornaviruses.

Contents

1. A brief history of the field

Borna disease (BD), 'Borna'sche Krankheit' in German (Gensert, 1896; Kohl, 1896; Schumm, 1896; Dexler, 1900) was first described as a meningoencephalitis of horses (Trichtern, 1716; von Sind, 1767; Abildgaard, 1795; Veith, 1822; Autenrieth, 1823; Wörz, 1858). The name Borna reflects outbreaks in the vicinity of the town Borna, in Saxony, wherein large numbers of animals died in the late 1800s (Königliche, 1896; Siedamgrotzky and Schlegel, 1896; Königliche, 1897, 1898, 1900; Schmidt, 1912; Zwick et al., 1926).

In the 1920s, transmission experiments between naturally infected horses and sheep, and rabbits, guinea pigs, rats, chickens, and monkeys, established the infectious nature of BD (Zwick and Seifried, 1925; Beck and Frohböse, 1926; Zwick et al., 1926, 1929; Nicolau and Galloway, 1928; Pette and Környey, 1935). Joest and Degen (1909) identified characteristic intra-nuclear inclusion bodies in the brains of animals with BD that provided the first diagnostic marker for disease and first clues to the unusual nuclear localization of the agent (Briese et al., 1992).

Interest in BD and its causative agent lapsed until the early 1970s when Rott, Ludwig and colleagues resurrected research on BD in Giessen and began to focus on identification of the agent and mechanisms of pathogenesis in rabbit, rat and tree shrew models (Ludwig et al., 1977; Sprankel et al., 1978; Narayan et al., 1983). In the early 1980s, Narayan's observations of a biphasic disease in adult-infected rats, characterized by initial hypermotility and excitability followed by depressed locomotion, led some investigators to suggest an analogy to bipolar disorder in humans (Narayan et al., 1983). This in turn prompted efforts to determine whether humans were infected with a related agent. Although Borna disease virus (BDV) was still uncharacterized, it had been propagated in primary tissue culture and transferred to permanent cell lines through co-cultivation (Mayr and Danner, 1972, 1974; Ludwig et al., 1973; Danner et al., 1978; Herzog and Rott, 1980),

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enabling the development of an immunofluorescence assay for serology (Wagner et al., 1968; Danner and Mayr, 1973; Ludwig et al., 1973). After a prominent publication from Rott and Koprowski in 1985 (Rott et al., 1985) reported that sera from patients with bipolar disorder were immunoreactive with infected cell lines, the authors of the present publication and others devoted themselves to identifying and characterizing the BD agent. Efforts to isolate virus for biochemical characterization or visualize particles by electron microscopy were unsuccessful in several laboratories. In the first application of purely genetic methods in pathogen discovery, cDNAs were obtained via subtractive hybridization and used to demonstrate relationship to disease through *in situ* hybridization experiments with rat brain (Lipkin et al., 1990). Thereafter, demonstration of the nuclear localization of transcription, RNA splicing and determination of the complete genomic sequence led to classification of BDV in 1996 (Pringle, 1996) as the first member of a new family *Bornaviridae* in the order *Mononegavirales* (Lipkin et al., 1990; Briese et al., 1992, 1994; Cubitt et al., 1994; de la Torre, 1994; Schneemann et al., 1994; Schneider et al., 1994). The identification of BDV sequences dovetailed temporally with the development of PCR as a tool for molecular epidemiology. Application of PCR, as well as serologic surveys, led to reports of BDV infection in association with a wide range of neuropsychiatric diseases. However, at the time of this writing, the question of human infection remains controversial. Molecular investigation of proventricular dilatation disease (PDD), a disease recognized primarily in psittacine species since the 1970s (Gregory et al., 1994), led in 2008 to the recognition of a virus that is genetically related to BDV, avian bornavirus (ABV) (Kistler et al., 2008; Honkavuori et al., 2008). ABV appears to be globally distributed (Rinder et al., 2009; Lierz et al., 2009; Weissenbock et al., 2009; Ogawa et al., 2011; Heffels-Redmann et al., 2011). The recent discovery of sequences distantly related to BDV L, M and N genes in the genomes of bats, elephants, fish, lemurs, rodents, squirrels, primates and humans (Horie et al., 2010; Belyi et al., 2010) indicates that at least historically, bornaviruses infected a wide range of vertebrate species.

2. Virion properties and molecular biology

2.1. Virion morphology

Spherical, enveloped particles ranging in diameter from 40 to 190 nm have been identified by electron microscopy in extracts from infected cultured cells (Zimmermann et al., 1994; Kohno et al., 1999). Particles of 90–100 nm or more contain a 50–60 nm electron-dense core and are presumed to represent infectious virions. Smaller particles are proposed to be defective. Spikes of 7 nm have been visualized on the larger particles that may represent the viral glycoprotein; however, this has not been confirmed by immunoelectron microscopy. To date, particles consistent with virions have not been identified in tissues or fluids from infected animals (Anzil and Blinzinger, 1972; Sasaki and Ludwig, 1993; Compans et al., 1994).

2.2. Genome organization

Complete genomic sequence has been reported for four equine isolates, strain V, HE/80, H1766, and No/98 (Briese et al., 1994; Cubitt et al., 1994; Nowotny et al., 2000; Pleschka et al., 2001); whereas strain V, HE/80, and H1766 sequences are 95% identical at the nucleotide (nt) level, No/98 sequence differs by more than 15% from the other three. The BDV genome is a linear, negative-stranded, nonpolyadenylated RNA comprising approximately 8900 nt. The genome is compact; 99.4% of its nt are transcribed into subgenomic RNAs. Only 55 of 8910 nt (strain V) are not found in primary viral transcripts. These nt represent the trailer region at the 5′-end of the genome (Fig. 1). The region between the 3′-end of the genome and the first base of the first transcriptional unit is 42 nt long and has a high adenosine/uridine content of 67%, similar to 3′-leader sequences of other non-segmented, negative strand (NNS) RNA viruses. Extracistronic sequences are found at the 3′ (leader) and 5′ (trailer) termini of the BDV genome that are complementary and have the potential to align to form a terminal panhandle. The genomic organization of avian bornavirus (ABV) is similar to that of BDV; however, homology between any ABV isolate and any BDV isolate is <70% at the nt level and <80% at the amino acid (aa) level (Honkavuori et al., 2008; Kistler et al., 2008). These differences notwithstanding, polyclonal antisera to the nucleoprotein and phosphoprotein of BDV are immunoreactive with ABV.

BDV has six major open reading frames (ORFs) (Briese et al., 1994; Cubitt et al., 1994) (Fig. 1) that code for polypeptides with predicted M_r of 40 kDa (p40), 23 kDa (p23), 10 kDa (p10), 16 kDa (p16), 57 kDa (p57) and 180 kDa (p190). Based on the positions of gene sequences in the viral genome, relative abundance in infected cells, and biochemical and sequence features, these polypeptides correspond to the nucleoprotein (N, p40), phosphoprotein (P, p23), matrix protein (M, p16), glycoprotein (G, p57) and L-polymerase (L, p190) found in other *Mononegavirales*. The p10 (X protein) does not have a clear homologue in other nonsegmented negative strand (NNS) RNA viruses (Wehner et al., 1997). It has been postulated to mediate nuclear shuttling of viral gene products such as unspliced RNAs and/or ribonucleoprotein particles (Wolff et al., 2002). It also is involved in the regulation of the viral polymerase (Schneider et al., 2003; Poenisch et al., 2004; Perez and de la Torre, 2005), appears to inhibit apoptosis (Poenisch et al., 2009), and the regulation of its expression may involve interaction of cellular proteins with its messenger RNA (Watanabe et al., 2009). The N protein contains a nuclear localization signal (NLS) as well as a nuclear export signal (NES), and is present in BDV in two isoforms (p40 and p38) that differ in length by 13 aa at the *N*-terminus; the NLS is located at the *N*-terminus of the p40 isoform. The P protein is acidic (predicted p*I* of 4.8), and has a high serine–threonine content (16%). Its phosphorylation at serine residues is mediated by both protein kinase C-ε and casein kinase II (Schwemmle et al., 1997; Prat et al., 2009). As with phosphoproteins of other *Mononegavirales*, P forms a central structural unit in the assembly of the active polymerase complex. P contains two NLS, binds to N, L and X, and may contribute through protein-protein interactions to nuclear localization of X and the 38-kDa isoform of N. The 16-kDa polypeptide is a putative matrix protein (Kraus et al., 2001). The ORF for p57 directs the synthesis of a glycoprotein of 94-kDa, a polypeptide that can be processed by the subtilisin-like endoprotease furin (Richt et al., 1998). Both GP-94 and its *C*-terminal cleavage product GP-43 are associated with infectious particles and are proposed to function in early events in infection (Gonzalez-Dunia et al., 1997, 1998). Incorporation of the *N*-terminal cleavage product GP-51 may also occur (Kiermayer et al., 2002; Eickmann et al., 2005). The ORF of BDV complementary to the 5′ half of the genome (L, p190) is fused to a small upstream ORF by RNA splicing to generate a continuous ORF with a coding capacity of 190 kDa in the 6.1 and 6.0 kb transcripts (Fig. 1). The deduced aa sequence of this ORF includes motifs that are conserved among NNS RNA virus L-polymerases.

2.3. Replication and transcription

Replication and transcription occur in the nucleus (Briese et al., 1992). Although this strategy is also found in some plant rhabdoviruses, it is a unique feature among animal NNS RNA viruses. In influenza virus, a segmented negative-strand RNA virus, the